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Manual of Bacterial Plant Pathogens

By Charlotte Elliott

Associate Pathologist, Bureau of Plant Industry, U.S.D.A.

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PHYTOPATHOLOGY

VOLUME 20 NUMBER 9

SEPTEMBER, 1930

A NEW BACTERIAL LEAF DISEASE OF TOBACCO IN THE PHILIPPINES[1]

FELICIANO M. CLARA[2]

A bacterial leaf spot of tobacco, resembling wild fire in some respects, was observed in December, 1925, in the seed beds and afterwards in the field of the Ilagan Tobacco Experiment Station of Isabela. Later, the same disease was found in several localities of the Cagayan Valley. The spots on seedlings generally are opaque and without the characteristic yellow halo of the wild-fire disease. On full-grown plants the yellow zone may be found at times associated with some spots. Due to the similarity of the disease to wild fire, it was at first regarded as such. The result of subsequent studies, however, indicated a disease different from wild fire and similar but not identical to that reported by Johnson (5) as "Wisconsin bacterial leaf spot" caused by *Phytomonas mellea* (Johnson) Bergey *et al.* Since the disease affects the quality and quantity of the crop produced, any authentic information about its occurrence, nature, and possible suppression will be of some value to growers.

DISTRIBUTION, ECONOMIC IMPORTANCE, AND LOCAL NAMES

The writer is not aware of any report of the occurrence of this disease in other parts of the world. It probably has been present in the Philippines but has been confused with other leaf spots of tobacco because of the names applied to it. The different local names given these diseases can not be used to distinguish one from the other. In the Cagayan Valley and the

[1] Published in Phytopathology with the permission of the Director of the Philippine Bureau of Agriculture. The greater part of this work was done at the Coöperative Plant Pathology Laboratory of the Bureau of Agriculture and the Bureau of Science, Manila.

The writer acknowledges the coöperation of Mr. G. Merino, Dr. C. J. Humphrey, Dr. N. G. Teodoro, Mr. D. B. Paguirigan, and Mr. F. B. Serrano in obtaining materials while conducting this investigation in the Philippines.

[2] I am particularly grateful to Professor M. F. Barrus and Professor W. H. Burkholder, who kindly corrected the manuscript and gave invaluable suggestions, advice, and encouragement while completing this work at the Department of Plant Pathology at Cornell University. Dr. Burkholder has also given me the privilege of consulting his manuscript on the genus Phytomonas, including several important references.

Ilocano regions of the northeastern part of Luzon, the commonest local terms employed for leaf spots, irrespective of the symptoms and causes, are "palatao," "palatae," and "batec." These names have no more significance, even locally, than has the term "leaf spot."

In the Cagayan Valley, the disease was particularly serious in 1925, and similar troubles seem to have occurred previously. It is very evident, however, that nothing was known about it. Specimens of fresh tobacco leaves received from Jaro Iloilo, one of the Visayan Islands, showed that the disease was present also in that region. A systematic pathological survey probably would show a much wider distribution and give a better realization of the rôle played by the disease in the tobacco industry.

The damage caused by the disease, independent of the other kinds of leaf spots, such as frog eye, wild fire, angular leaf spot, and others, merits some attention. The extent of infection on seedlings in the seed beds of the Ilagan Tobacco Experiment Station during 1925 and 1926 and later transmitted to the field caused great concern. Seedlings grown in seed flats, although the soil employed was sterilized with formaldehyde, showed from 50 to 90 per cent damage. Presumably, the same trouble occurred in the seed beds of individual growers, as there was a great shortage of seedlings during that season. When infected plants were set in the field, a large percentage of the leaves at harvest showed the effect of the disease. Some were rendered nearly useless, while others fell into an inferior and cheaper grade. The damage varies according to the use made of the different classes of leaves. Spotted wrapper leaves can not be used for high-grade cigars because of their lack of uniformity in color and their poor texture. Such leaves can be used only as binders and fillers. But, even in such cases or however used, the disease lowers the quality of the product. The real effect upon the quality of the crop, in addition to other losses, does not receive proper recognition on account of the system of marketing in vogue, whereby no discrimination is made by the buyer. This practice gives no encouragement to produce a good quality of tobacco which can be brought about, other things being normal, through the control of disease. As to the actual reduction in the quantity of the crop, no estimate was made. There is, however, no doubt that the disease is a limiting factor, as shown by its seriousness both in the seed beds and in the fields.

SYMPTOMS OF THE DISEASE

In the seed beds. Young seedlings when severely infected develop a kind of wet-rot similar to bed rot (Fig. 1, A and B). The young plants may thus be wiped out and the condition easily confused with typical damping-off diseases. The presence of opaque or bleached white spots on

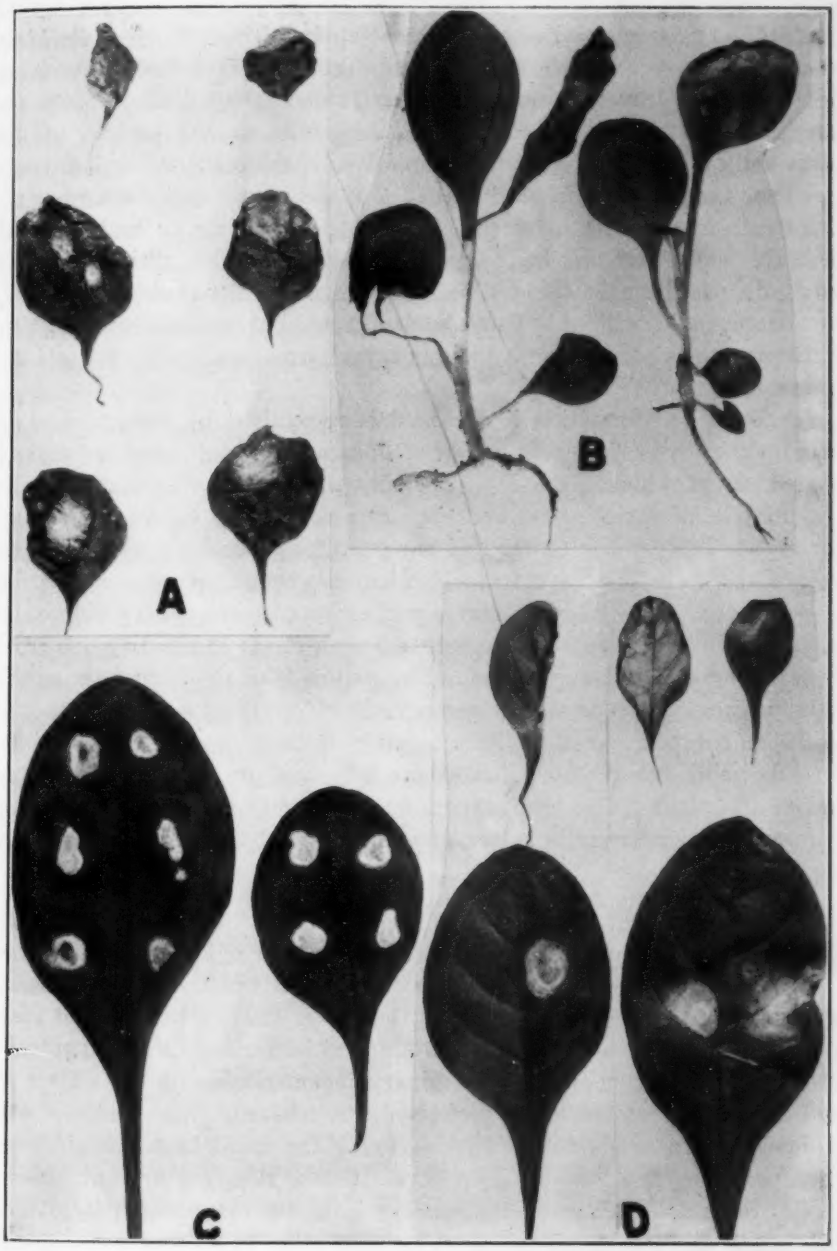

FIG. 1. A. Natural infections produced by **Phytomonas polycolor,** n. sp., on leaves of tobacco seedlings. B. Wet-rot form produced by inoculations through needle punctures. C. Inoculations with bacterial smear without punctures. D. Inoculations with bacterial smear through needle punctures. Natural size.

the leaves (Fig. 1, C and D) shows that the disease is actually different from ordinary damping-off or bed rot associated with such fungi as *Pythium debarynum* Hesse and *Sclerotium rolfsii* Rach. When infection starts on the edge of the leaves, a wet-rot becomes evident (Fig. 1, B). More apparent damage results when the infection occurs on the petioles of the leaves and on the stems, as it then rapidly kills the plant. The leaf spots vary from the size of a pin head to that of a "centavo," and there are cases where entire leaves are involved. The spots are visible on both sides of the leaves and sometimes holes are produced. Wild fire, which may also be found on seedlings in the seed beds as well as on older plants in the field, may be confused with this bacterial leaf spot, but a closer examination of the symptoms and characters of the causal organism readily reveals the distinction.

In the fields. Infection in the field is recognized by the presence of spots on the leaves, generally white or opaque, but sometimes brown and zonated, about which there is occasionally a narrow yellow halo. These spots may be of various sizes and may even coalesce to form larger irregular ones. The grayish center and the pustules generally present in frog eye are absent in these spots except when a secondary organism has developed there. Young lesions may occasionally possess a yellow halo quite similar to wild fire. In such cases it is very difficult to identify one from the other by external appearance. The yellow "halo" disappears as the leaves mature. The disease is more virulent on the lower than on the middle and upper leaves, possibly because of their greater susceptibility or to the more favorable conditions for infection proximate to the lower part of the plant. The brown spots are not easily seen on cured leaves because of the similarity in color to such leaves, but the white ones remain conspicuous.

CAUSE OF THE DISEASE

Isolation. The causal organism, among a number of other bacteria, was first isolated from diseased seedlings in the seed beds of the Ilagan Tobacco Experiment Station at Isabela during December, 1925. Hundreds of such isolations were made and the different kinds of bacteria obtained in culture were numbered and used for preliminary inoculations with the object of eliminating all that were not pathogenic to tobacco. Each culture was obtained from a single colony by employing the usual bacteriological dilution procedure in all the isolation work. One of these cultures, numbered 116-C31, which showed green fluorescence in broth, was found very pathogenic, while the others did not show any infection at all and were, therefore, discarded. This organism was later repeatedly isolated from freshly infected leaves of full-grown plants, from badly spotted leaves in the

curing sheds of the Experiment Station in 1925 and 1926, and from leaves received from Iloilo in 1927. It was obtained also from leaves that had been dried between papers and kept in the laboratory for more than a year. Some isolations made from seedlings that had developed a wet rot, typical of the disease, showed this same organism. These seedlings came from seeds that were germinated, without disinfection, on absorbent cotton in petri dishes and kept moist with sterile water. This seems to indicate the presence of the pathogen on the seeds.

The material from which isolations were made was first placed in 1-1000 mercuric chloride for 10 to 20 seconds and then rinsed several times in sterile water. It was then dropped in beef bouillon, +1 Fuller's scale, from which dilution plates were prepared the third day. Plating was made on potato-dextrose agar, +1. This gave an abundant growth after 24 hours under room condition and proved the best medium for obtaining a sufficient supply of inoculum.

Inoculations. Several inoculation experiments were made on seedlings and on plants at various ages up to the flowering stage. These plants were all grown on sterilized soil and kept in a building covered with wire mesh. Culture 116-C31, on potato-dextrose agar, +1, except in a few cases, was used for all of the inoculation experiments.

Various methods were employed, such as inoculating in punctures made with a sterile needle, spraying the host with a bacterial suspension in distilled water, and direct application of a bacterial smear.

When inoculations of seedlings were made in punctures and the pathogen applied in water-drop suspension, the infection was very rapid and frequently developed into a wet rot (Fig. 1, B). Even quicker results were obtained when bacterial smear was applied to the punctures with a 3 mm. platinum-wire loop. The infection was readily noticed after twelve hours, especially if the plants were kept under bell jars. Practically all parts of the plant—the stem, the petiole of the leaves, the youngest shoots—were easily infected, finally resulting in the death of the entire plant. Inoculation with bacterial smear, with and without punctures, readily showed infections (Fig. 1, C and D). The check plants, although punctured and placed under the same conditions, except the application of the inoculum, remained normal.

On nearly full-grown plants and at other stages, inoculations made by applying a smear of the culture to the leaves and locating them where desired to facilitate detection of the infection proved to be satisfactory for comparative purposes (Fig. 2).

In all cases, both inoculated and control plants were kept under bell jars from 12 to 24 hours. The infection was noticeable on the inoculated points on the second day, and the spots were invariably opaque or white.

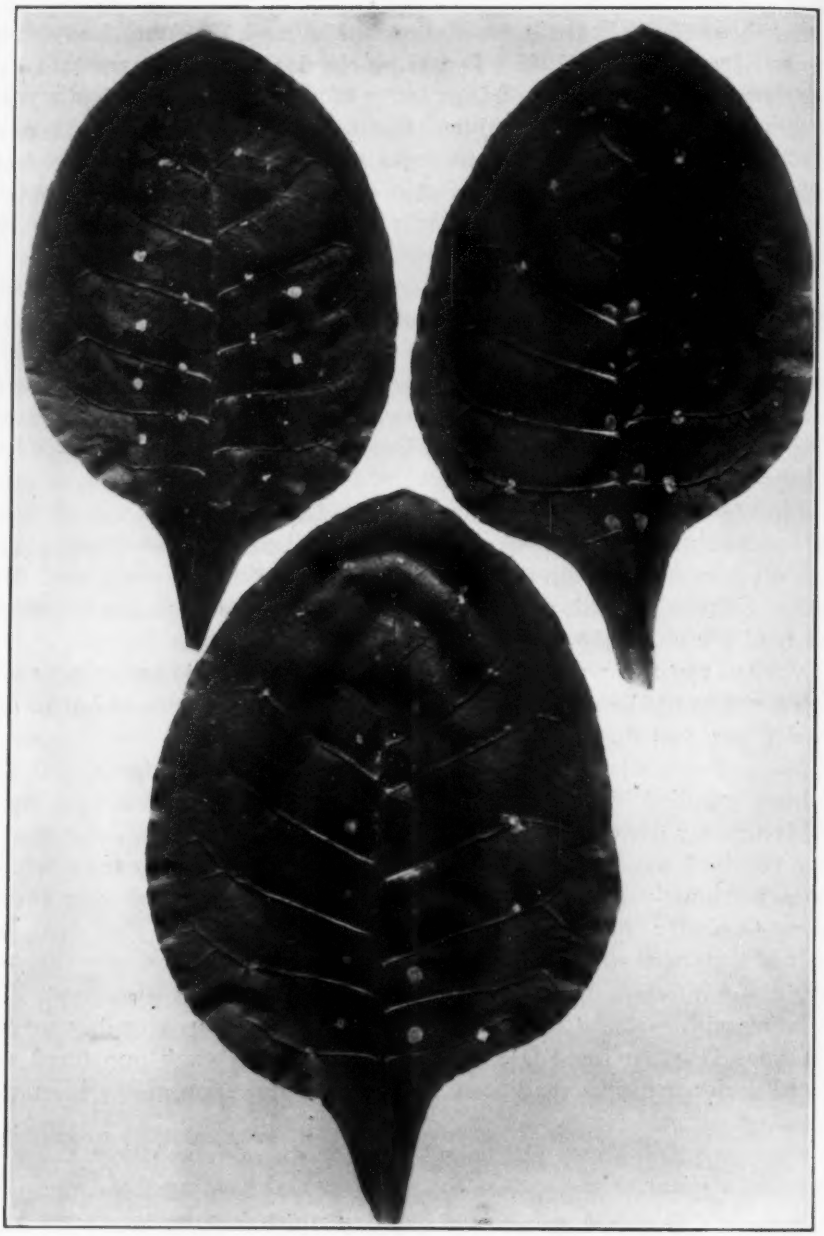

FIG. 2. Results of inoculations with bacterial smear of **Phytomonas polycolor**, n. sp., applied without injuring the surface, on several parts of the leaves. About ½ natural size.

TABLE 1.—*Results of inoculations of upper, lower, and middle leaves of different varieties of tobacco with the bacterial leaf-disease organism*

Variety name	Age of suscept	Method of inoculation	Leaves inoculated	Number of inoculations	Per cent infection after		Reisolation
					4 days	14 days	
Tall Zimmer	3 mo. 23 da.	Culture in beef bouillon	Upper	180	17	17	+
" "	" "	Inoculation through punctures	Middle	180	25	25	+
" "	" "	" "	Lower	180	50	50	+
" "	" "	" "	Upper	60	0	0	−
" "	" "	" "	Middle	60	5	5	+
" "	" "	" "	Lower	60	20	20	+
Baker Sumatra	3 mo.	Bacterial smear	Upper	123	43	46	+
" "	"	" "	Lower	462	68	71	+
P. I. 9352 SP. No. 3	4 mo.	" "	Upper	40	48	48	+
" "	"	" "	Middle	40	62	62	+
" "	"	" "	Lower	40	100	100	+
N. T. Havanensis	"	" "	Upper	20	20	20	+
" "	"	" "	Middle	20	30	30	+
" "	"	" "	Lower	20	50	50	+

When the same method of inoculation was used under field conditions the amount of infection was comparatively less and showed up more slowly, obviously because of the less favorable environmental factors. What these favorable factors are, however, is not well understood.

Several inoculations made by spraying the suscept with a water suspension of the bacteria gave a few positive results. When cultures in beef bouillon were used as inoculum comparatively few infections were obtained.

A difference was observed in the appearance of the spots produced by the application of a bacterial smear to punctures and those obtained by the application of the smear to the uninjured surface. The disease was readily produced by both methods, but when the smear was applied to the punctures the resulting spot occasionally developed a yellow halo around the lesion. This halo was especially noticeable during the early stage of the infection, but disappeared with age, when the spots became white or sometimes brown. When the latter method was employed the spots were invariably opaque. Table 1 summarizes the results of these inoculations.

A very marked difference in susceptibility among the lower, middle, and upper leaves can be noticed. The lower leaves are the most susceptible, showing in some cases 100 per cent infection; the middle ones ranked second; and the youngest were more rarely infected. Leaves that have been allowed to become mature or overmature are readily infected. Nearly all parts were used for inoculation, but the infection showed much more readily when the inoculations were made between the leaf veins. When the inoculum was placed exactly on the veins, the infection appeared in some cases at both sides, as if the pathogen were unable to infect the veins directly. In other cases, spots developed as on other points on the leaves.

EVIDENCES OF VARIETAL SUSCEPTIBILITY

An examination of the results of the inoculations shows a different degree of infection on each of the four varieties of tobacco employed in the study. Considering only the upper and lower leaf infections, it can be seen that the Tall Zimmer and N. T. Havanensis showed an average of 33.5 per cent and 37.5 per cent, respectively, while Baker Sumatra showed 61.40 per cent and P. I. 9352 S. P. No. 3 showed 73.75 per cent. The two latter varieties had about twice the percentage of infection as the two former ones. The varietal differences in leaf texture may be correlated with degrees of susceptibility. The Sumatra varieties are wrapper types and possess comparatively thinner leaves than the other varieties when grown under suitable condition. Further studies along this line are, however, necessary before a definite conclusion can be made.

FIG. 3. A. Streak culture on potato-dextrose agar showing the filiform growth widening at the bottom. B. Submerged and surface colonies on potato-dextrose agar. Natural size. C. Bacteria, single and in chains, stained with carbol fuchsin. ×1560. D. Bacteria showing flagella, stained with Plimmer and Paine's stain. ×1560.

THE PATHOGEN

Morphology. The organism is a motile rod with rounded ends; single or in pairs; mono- or lophotrichous (Fig. 3, D). Measurements of stained mounts obtained from 24-hour-old culture on potato-dextrose agar, +1, ranged from 1.19 to 4.12 μ in length by 1.06 to 1.33 μ in width. The average size is 2.68 by 1.27 μ. The organism is easily stained with carbol fuchsin, methylene blue, aniline gentian violet, and aqueous gentian violet. Plimmer and Paine's stain (6) for flagella gives very good results with 27-hour-old cultures on potato-dextrose agar, +1, at temperatures 18° to 20° C. One to two flagella, usually one, are observed. They are spiral-like and about two to three times longer than the body of the organism (Fig. 3, C and D). The organism is gram-negative and is not acid-fast.

Cultural characteristics.—Potato-glucose agar, +1. The organism grows rapidly on this medium. In poured plates the colonies appear on the second day. They are colorless at first, but begin to turn yellow on the third day. In shape they are convex, glistening, with entire margin. Submerged colonies are lenticular and small (Fig. 3, B). On long standing, surface colonies become olive brown. Streak cultures are grayish white to Primuline Yellow[3] at room temperatures, 25° to 30° C. (Fig. 3, A). A yellowish growth is produced at the bottom of some of the cultures. Some of these yellow growths on more than year-old cultures were still viable and pathogenic, while others four months old did not grow at all. Why there was such a difference is not well understood and no attempt was made to study the underlying causes.

In the course of continuous culturing of the organism, 116-C31, three variants developed in some of the stock cultures on potato-glucose agar, and on potato plugs more than a year old. At first it was thought that these seemingly extraneous growths of the cultures were contaminations. Subsequent morphological, physiological, and biological studies, however, showed that none of them fell under this category. The first, type 116g-C31, is characterized by a bluish green color which may or may not be imparted to the medium. Colonies develop green granular pigments at the center in three days under room condition. The green color disappears with age and some yellowish growth develops on the surface. The culture, however, remains viable for a long time. The second subtype (116p-C31) is characterized by the production of pink lavender turning to purple with age on lead-acetate agar. The third subtype, labeled 116w-C31, is a rapid-growing, grayish white organism having a filiform growth on a slant of potato-dextrose agar, +1, and living only for a short time.

[3] The color names beginning with capitals are those of Ridgway's Color Standards and Nomenclature. Washington, 1912.

Any of these subtypes were readily obtained from colonies either from the yellow type (cul. 116-C31), from the subtype colony with green centers (116g-C31), or from subtype pink lavender colony (116p-C31) on poured plates of suitable media. Although no extensive detailed study of this behavior of the organism other than on solid media, broth, and sugar solutions was made, a certain degree of biological and physiological differences was noted. The subtype 116g-C31, for instance, seemed to remain viable longer in culture under ordinary room conditions than all other cultures. Some variability in the virulence of the subtypes has also been observed. The whole phenomenon finds explanation in bacterial dissociation so ably discussed by Hadley (3).

Beef-peptone agar. Growth along the streak, flat filiform, slightly widening at the bottom, smooth, glistening, fluorescent on the third day but becoming nonfluorescent within seven days, the medium becoming Marguerite Yellow.

Glycerine agar. On this medium a profuse filiform growth, grayish white, smooth, and glistening is produced. An Opaline Green color is produced and imparted to the medium. On long standing the medium becomes greenish brown and a Sulphur Yellow filiform growth on the surface is produced. The organism remains viable on this medium for some time.

Potato cylinder. The growth spreads out, at first yellow glistening and becoming sepia brown. The organism lives long on this medium. Five months and fourteen days under room temperatures showed viable transfers on potato-glucose agar.

Potato-agar slant. A rather poor, colorless to grayish white, filiform, and slightly raised growth develops after the lapse of one day.

Beef bouillon + 1. Growth is very noticeable after 16 hours but is confined to a few centimeters near the surface, indicating strong aërobism. A fragile, flaky pellicle is present on the surface. Green fluorscence appears in the turbid region. On older cultures a white precipitate settles to the bottom.

Gelatine stab. At 18° to 20° C. no liquefaction is observed until the fourth day; at first crateriform, becoming stratiform on the sixth day; the medium completely liquefies in one to four weeks. At 7° to 9° C., liquefaction is very slow. Under this condition no liquefaction appears until after 23 days. The medium becomes completely liquefied and a whitish-gray precipitate within 3 months settles to the bottom.

Loeffler's blood serum. A sunken furrow-like, filiform growth is very noticeable after 16 hours. Frequently the medium cracks and liquefaction starts on the third day, when a distinct disintegration is noticed, generally accompanied by the breaking down of about two-thirds of the slant.

Litmus milk. Apparent change is observed on the third day when a creamy-white to yellow ring is formed around the glass about the surface of the liquid. The liquid becomes Cinnamon Buff, indicating a gradual reduction of litmus starting from the upper portion and continuing gradually towards the bottom. Reduction is complete in twenty-six days and various color changes occur during the process. Neither curdling nor peptonization is produced but a colorless deposit settles to the bottom. The medium became alkaline.

Starch. Starch is not digested.

Beef-agar slant. Growth scanty and noticeable after 16 hours. Grayish white at first, later turning yellow; much less profuse than on potato-dextrose agar and on potato plug.

Indol. No indol is produced on tryptophane broth. Erlich-Böhme technic (7) was employed in the test.

Nitrite in nitrate-peptone broth. Nitrate was reduced to nitrite in nitrate-peptone broth (Merck's nitrite-free reagent). Harper's prenol-disulphonic-acid method (4) for nitrite detection was employed. Johnson did not obtain nitrate reduction with *Phytomonas mellea.*

Litmus-sugar agars. Glycerine, glucose, lactose, and saccharose agars were used. No litmus reduction was obtained by Johnson (5) in any of these sugars with *Ph. mellea,* while, with the Philippine bacterium, litmus is reduced in glucose agar four days after inoculation. The medium becomes Ox Blood Red from the upper portion, extending gradually towards the bottom. In glycerine, lactose, and saccharose, acid is not produced.

Fermentation. The use of Dunham's 2 per cent peptone water resulted in the production of no gas on 1 per cent solutions of the following carbohydrates: xylose, arabinose, levulose, mannose, galactose, glucose, lactose, maltose, saccharose, raffinose, rhamnose, inulin, glycerine, mannite, sorbite, dulcite, inocite, dextrin, salicin, amygdalin, and adonite. For the detection of acid formation, Andrade's indicator was employed. Strong clouding, denoting the growth of the organism, is observed in all cultures 16 hours after inoculation. Clouding invariably appears near the surface of the liquid associated with various shades of fluorescence. Acid formation is noted in xylose, arabinose, glucose, and mannose.

Hydrogen sulphide. Cultures on basic lead-acetate agar show the production of hydrogen sulphide.

Physiological reaction—Temperature relations. At temperatures 7° to 9° C. the organism grows very slowly, at 20° to 24° the growth is moderately profuse, and at 37° to 39° the growth is most profuse, being 8 mm. wide at the bottom in 16 hours, but no viable transfers were obtained after a short time. Gray spots appear on the strokes and no pigmentation.

Johnson (5) did not obtain any growth with *Phytomonas mellea* at 35° to 36° C.

Resistance to desiccation. Drops of beef-bouillon culture placed on sterilized cover glasses kept in sterilized petri dishes are still viable after six days but show no growth after 12 days.

Viability and virulence. The organism grows well on potato-glucose agar, + 1, and remains alive for a long time. Viable transfers are obtained from some cultures more than one year old. Potato plugs and glycerine agar maintain equally well the viability of the organism with no sign of loss of virulence.

Nomenclature. A comparison of this organism with all other known bacteria causing disease of tobacco and of the symptoms produced shows that it resembles *Phytomonas mellea* more than any of the others. No comparative study of the two organisms was made, as no culture of *Ph. mellea* could be obtained. But a comparison of the characteristics of the Philippine organism with those of *Ph. mellea* as described by Johnson (5) presents very significant divergences which would not logically place the Philippine bacterium in the same species. The symptoms produced, the morphology of the organism, reaction in carbon media, reduction of nitrate into nitrite, and action upon milk and starch are very different from those of *Ph. mellea.*

TABLE 2.—*Characteristic differences between Phytomonas mellea and the Philippine organism*

Phytomonas mellea	*Philippine organism*
1. Brown "rust" is produced	1. White spot is produced
2. Chlorotic halo frequently present	2. Chlorotic halo very rarely present
3. Average size of bacterium, 1.8 by 0.6 μ	3. Average size of organism, 2.68 by 1.27 μ
4. One to seven polar flagella	4. One to three polar flagella
5. Growth on potato-dextrose agar, Honey Yellow to deep orange	5. Growth on potato-dextrose agar, Primuline Yellow, fluorescent
6. On potato cylinder, growth brownish yellow	6. On potato cylinder, growth yellowish to sepia brown; bluish green color exhibited at 20° to 22° C.
7. Starch digested	7. Starch not digested
8. Litmus milk becomes alkaline, coagulated, and slowly peptonized	8. Litmus milk rendered alkaline but not curdled nor peptonized
9. Nitrate in nitrate broth, no reduction into nitrite	9. Nitrate in nitrate-peptone broth reduced into nitrite
10. No acid formed in glucose, lactose, and saccharose broth	10. Acid formed in glucose, mannose, xylose, and arabinose but not in lactose and saccharose; fluorescence exhibited

According to Johnson (5), the species *mellea* is so named because of the development of a honey color in culture. It peptonizes milk, digests starch,

and does not reduce nitrate. The Philippine bacterium does not peptonize milk nor digest starch. It reduces nitrate to nitrites and produces a characteristic green fluorescence of various shades in nearly all cultures, particularly in carbon and broth media. Colonies with green centers also are produced. This suggests at once that the organism belongs to the fluorescent group, the plant pathogens of which Bergey *et al.* (1) include in the genus Phytomonas. Burkholder (2), however, in "The Genus Phytomonas," allocates this group to the genus Pseudomonas Migula to include all distinctly green fluorescent bacteria, based upon their logical and significant natural relationship.

Among this group of bacterial pathogens the species that approach it in similarity, although distinctly not identical to it, are *Phytomonas Tolaasi* (Paine) Bergey *et al.*, parasitic on cultivated mushrooms, *Ph. bowlesia* Lewis and Weston, parasitic on *Bowlesia septentrionalis; Ph. martyniae* (Elliott) Bergey *et al.*, parasitic on *Martynia louisiana;* and *Ph. marginalis* (Brown) Bergey *et al.*, parasitic on lettuce. All these species, however, differ from the one studied in their ability to curdle and peptonize milk. The latter two differ also by fermenting sucrose. *Ph. marginalis* differs further in its inability to produce hydrogen sulphide. There may be other species in the group which present more or less similarity to the Philippine organism, but this could not be ascertained because of the present uncertainty or absence of knowledge of their physiological characters. Another very distinctive characteristic of the organism is its ability to produce intensely green pigments on glycerine agar and a pink lavender color, which turns purple, on lead acetate agar. However, some cultures on lead acetate agar behave as on glycerine agar.

It is thus very clear that the organism can not be identified with any of the previously described members of the green fluorescent group nor with any other known bacterial plant pathogen.

TECHNICAL DESCRIPTION

Phytomonas polycolor, n. sp.[4] Rod-shape type with rounded ends; occurring singly in chains and in pairs; average size 2.68 by 1.27 μ; motile by mono- or lophotrichous, one to three flagella but generally one, about two to three times longer than the body; no spores nor capsules were observed; gram-negative and not acid-fast; readily stained with carbol fuchsin, methylene blue, aniline gentian violet, and aqueous gentian violet; a facultative anaërobe and markedly fluorescent.

[4] Bergey *et al.* limited the generic name Pseudomonas Migula to the nonphytopathogenic fluorescent bacteria. However, if the term is applied to all green fluorescent bacteria, as proposed by Burkholder (2), the name of this organism should be *Pseudomonas polycolor,* n. sp.

Agar colonies, convex with entire transparent peripheral margin; colonies producing green centers develop from old cultures; submerged colonies, small and lenticular; streak growth on potato-glucose agar, abundant, raised, widening at the bottom, glistening, smooth and slightly viscid; green fluorescence in some cultures; in nutrient broth, heavy clouding with fluorescence starts from the top, producing delicate flaky membranes; on potato plug, growth spreading, yellowish, becoming sepia brown with age; litmus milk is reduced and rendered alkaline but does not curdle nor peptonize milk; starch is not digested; xylose, arabinose, glucose, and mannose are fermented, producing acid reaction but no gas formation; in other solutions, such as lactose, saccharose, and other sugars used, no fermentation; gelatine and Loeffler's blood serum, liquefied; nitrate reduced to nitrites; no indol production; produces hydrogen sulphide on basic lead acetate agar; optimum temperature, within 25° to 30° C., maximum temperature, 37° to 39° C. The organism is pathogenic on tobacco, *Nicotiana tabacum* L.

CONTROL MEASURES

Seedling infections have always been observed to be carried to the field. Attempts to show seed-borne infection were made by collecting and planting seeds from badly infected plants showing lesions on the capsules. Some of these seeds were planted under bell jars and others under ordinary condition. The disease appeared on the seedlings under both conditions. The organism obtained from these plants was as pathogenic as the original stock culture. Because of this evidence, seed-disinfection tests with silver nitrate, 1-1000, and mercuric chloride, 1-1000, in separate treatments for 10 to 15 minutes, were made as recommended in the case of other bacterial leaf diseases, *i.e.*, wild fire and angular leaf spot. The seeds were rinsed carefully several times with distilled water and dried in the room before sowing. These tests were tried out during two successive years, 1925 and 1926. The former disinfectant gave more encouraging results both in the reduction of the disease and less untoward effects upon the rate of germination. It seems, from the results obtained thus far, that silver nitrate would be more suitable than mercuric chloride for tobacco-seed disinfection under Philippine conditions.

SUMMARY

1. A new bacterial leaf disease of tobacco, characterized by white or opaque spots, has been under observation in the Cagayan Valley and on specimens received from Iloilo during a period of more than two years.

2. The nature of the damage is a reduction in the quality and quantity of the crop produced. Seedlings may also be destroyed entirely or ren-

dered unsuitable for transplanting. The exact extent of losses and the distribution of the disease are not well known.

3. The causal organism of the disease is a bacterium hitherto unknown and is named **Phytomonas polycolor**, n. sp. A description of the organism is given.

4. Artificial inoculations have conclusively shown the pathogenicity of the organism and the reproduction of a disease identical to that found in nature.

5. The damage from the disease seems to be reduced by seed disinfection with silver nitrate, 1-1000, for 10 to 15 minutes. This method serves to destroy other seed-borne organisms, *i.e.*, those causing wild fire and angular leaf spot.

COÖPERATIVE PLANT PATHOLOGY,
BUREAUS OF AGRICULTURE AND SCIENCE,
MANILA, P. I.

LITERATURE CITED

1. BERGEY, D. H., *et al.* Bergey's manual of determinative bacteriology. Ed. 2. Williams and Wilkins Co., Baltimore. 1925.
2. BURKHOLDER, W. H. The genus Phytomonas. Phytopath. 30: 1–24. 1930.
3. HADLEY, PHILIP. Microbic dissociation. The instability of bacterial species with special reference to active dissociation and transmissible autolysis. Jour. Infect. Dis. 40: 1–312. 1927.
4. HARPER, H. J. The accurate determination of nitrates in soils. Phenol disulfonic acid method. Indus. and Engin. Chem. 16: 180–183. 1924.
5. JOHNSON, JAMES. A bacterial leafspot of tobacco. Jour. Agr. Res. 23: 481–494. 1923.
6. PLIMMER, H. G., and S. G. PAINE. A new method for the staining of bacterial flagella. Jour. Path. and Bact. 24: 286–288. 1921.
7. Society of American Bacteriologists, Committee on Bacteriological Technic. Manual of methods for pure culture study of bacteria. 48 pp. Geneva, N. Y. [1923]. 1926.

INHERITANCE OF IMMUNITY FROM FLAX RUST[1]

A. W. HENRY[2]

INTRODUCTION

The inheritance of many of the morphological characters of the flax plant has been carefully treated by Tammes (7), Davin and Searle (2), and others. Few studies, however, have been reported on the genetics of disease resistance in flax. Tisdale (8) investigated wilt caused by *Fusarium lini* Bolley and concluded that resistance to this disease was determined by multiple factors.

Results are presented in the present paper of studies on the inheritance of immunity from flax rust, caused by *Melampsora lini* var. *liniperda* (Pers.) Lév., made during the years 1923 to 1926. A brief report is also given of progress made during this period in breeding improved rust-immune varieties of seed and fiber flax.

Most of the varieties now grown in North America are susceptible to rust. Some of the wilt-resistant seed flaxes now in use are very susceptible to rust. Since this disease occasionally causes losses as high as ten per cent in yield of seed, it is desirable that new varieties should be immune from rust as well as resistant to wilt. Rust also has been introduced into the fiber-flax centers of North America. The disease is especially important to fiber-flax production since the stems may be rendered worthless for fiber purposes if severely attacked. The introduction of immune varieties would remove this danger. Having found several varieties of seed flax which are immune from rust (4), it was thought possible to transfer this quality into taller varieties suitable for fiber purposes.

PARENTAL MATERIAL

The following rust-immune parents were used in these studies: Argentine selection, Ottawa 770B, and Bombay. Several immune parents were used, as each possessed certain valuable characters in addition to immunity from rust. Moreover, owing to the possibility of forms of rust becoming prevalent that would attack one of these varieties, it was thought advisable to use several immune parents in the crosses.

[1] Contribution from the Department of Plant Pathology, Minnesota Agricultural Experiment Station, St. Paul, Minn. Cooperative investigations between the Minnesota Agricultural Experiment Station and the offices of Cereal Crops and Diseases and Fiber Plants of the U. S. Department of Agriculture.

[2] Formerly Assistant Plant Pathologist of the Minnesota Agricultural Experiment Station and Agent, U. S. Department of Agriculture; now Assoc. Prof. of Plant Pathology, University of Alberta, Edmonton, Canada.

All three varieties have been thoroughly tested with different collections of flax rust and have remained immune in all tests made by the writer and in all tests known to him. A few susceptible plants have been found in Argentine selection and Bombay, but these quite evidently were mechanical mixtures or the result of natural crossing, as the bulk of the plants proved immune. Tests have been made with several American collections of flax rust from both the United States and Canada, as has been previously reported (3). Argentine selection and Ottawa 770B, together with several susceptible varieties, such as Winona and Saginaw, have been tested by Hiratsuka (6) in Japan, with the same results as ours. Argentine selection and Ottawa 770B were also immune from the Japanese forms of this rust. During the summer of 1927, while in Europe, the writer inoculated these three varieties with a collection of flax rust kindly supplied by Dr. Tine Tammes from her flax-breeding garden at the University of Groningen, Groningen, The Netherlands, and all three remained immune. Their choice as parents from the standpoint of their reaction to rust seems, therefore, to have been a fortunate one.

The above parents were obtained originally from the following sources: Argentine selection, from commercial Argentine flax obtained from the Pittsburgh Plate Glass Company of Red Wing, Minnesota, and the original selections made by Dr. H. D. Barker, formerly of the Minnesota Agricultural Experiment Station and U. S. Department of Agriculture; Ottawa 770B, from Mr. R. L. Davis, formerly in charge of fiber-flax breeding for the U. S. Department of Agriculture at East Lansing, Michigan, who had previously received it from the Dominion of Canada Department of Agriculture; and Bombay C. I. 42, kindly provided by J. C. Brinsmade, Jr., of the Northern Great Plains Field Station of the U. S. Department of Agriculture at Mandan, North Dakota.

Argentine selection, in addition to being immune from rust, is also highly wilt-resistant. This has been shown by several years tests on "sick" soil at University Farm, St. Paul. It is a large, brown-seed, late-maturing type of medium height, with large blue flowers. The stems, however, are too short and coarse and branch too freely to be of value for fiber purposes. Numerous selections of this type of flax, obtained from different commercial lots of Argentine flax, have thus far been immune from rust (3).

Ottawa 770B is a medium-size, yellow-seed variety with small white crimpled flowers. It matures too late and yields too little seed to be used as a seed flax. Although somewhat taller than Argentine selection, it is not tall enough nor fine enough in the stem to make a good fiber variety. Bombay is a medium-size, brown-seed variety having small blue flowers. It is a short, early-maturing type. It is too short, however, to become of impor-

tance as a seed flax in the seed-flax areas of the United States and is naturally also unsuitable as a fiber variety.

The susceptible varieties chiefly used in these studies were Saginaw and Winona. Saginaw is a very tall variety of fiber flax developed by the Office of Fiber Plants of the U. S. Department of Agriculture. It has small brown seeds, small bolls, and small blue flowers. It has long, fine stems but produces very little seed. It is quite susceptible to rust, though it usually does not develop as heavy infections in the later stages as does Winona. In maturity it is relatively early for so tall a variety. Winona is a medium-early variety of seed flax developed at the University of Minnesota by the Departments of Plant Pathology and Plant Breeding. It was selected particularly for its excellence in wilt resistance, from Blue Dutch Minn. 175 (1). It has small, brown seed and small, dark blue flowers. Though one of the best wilt-resistant seed flaxes, it is very susceptible to rust. Two other varieties of seed flax similar to Winona, namely, Blue Blossom Dutch and Chippewa, were used as parents in some of the crosses. The former was obtained from the U. S. Department of Agriculture, while the latter is another wilt-resistant selection produced by the Minnesota Agricultural Experiment Station, being a selection from Primost, Minn. 25 (1).

METHODS

Most of the crosses were made in the field at University Farm, St. Paul, Minnesota, during the summer of 1923.[3] For the most part, the emasculated flowers were pollinated immediately and covered with cigarette tubes fastened with small paper clips and placed close to the flower pedicels and parallel to them.

Argentine selection was crossed with Saginaw, Winona, and Chippewa, but the inheritance of immunity from rust was not studied in detail in the crosses with the two latter varieties. In most of the Argentine selection by Saginaw crosses, Argentine selection was used as the female parent, since it sets seed better than Saginaw and since its large flowers are easier to manipulate. However, the reciprocal cross also was made. Ottawa 770B, as the male parent, was crossed with Saginaw and Winona. Bombay was used as the male parent in crosses with Winona, Blue Blossom Dutch, and Saginaw. Detailed inheritance studies were made only on the first two of these crosses. Some of the data were obtained from greenhouse inoculations; for instance, the F_1 of the Argentine selection by Saginaw crosses. This was grown in the greenhouse during the winter of 1923–24. Artificial

[3] A few crosses were made by H. D. Barker in the greenhouse in the winter of 1922–23 between Argentine selection and Saginaw. Five hybrid seeds were obtained from these crosses.

light was used at night in order to hasten the maturity of the plants. They were first inoculated with rust in the seedling stage by applying urediniospores to the cotyledons and leaves by means of a flat inoculating needle. They were inoculated a second and a third time by spraying with a suspension of urediniospores in water as well as by dusting with dry urediniospores from infected plants. Part of the F_2 of this cross as well as plants from a back-cross of the F_1 on Saginaw were also inoculated in the greenhouse and treated in a manner similar to that employed with the F_1. Parental plants also were inoculated to serve as checks with each set of inoculations.

Data on the rust reaction of the remainder of the F_2 and of the F_3 of the Saginaw × Argentine-selection cross, together with all generations of the crosses in which Bombay and Ottawa 770B were the immune parents, were obtained in the field. The rust nurseries where this material was tested were sown on peat soil at Coon Creek, Anoka County, and Clearspring, Hennepin County, Minnesota.[4]

It was found advisable to use the peat bogs for these nurseries, since epiphytotics were readily obtained there each year, whereas on higher land they could not always be obtained with certainty. The nurseries were artificially inoculated in order to insure early and uniformly heavy infections. The variety Winona, especially susceptible to rust, was largely grown for borders and intervening rows in the nurseries on which to increase the rust. Plants heavily infected with urediniospores were brought from the greenhouse, and the rust was dusted or sprayed on the plants in the field until a uniform epiphytotic was obtained. Old teliospore-bearing straw also was spread over the nurseries in the spring or early summer in some cases.

RUST REACTION OF THE F_1

F_1 plants of all crosses were inoculated with rust either in the field or in the greenhouse. The result was the same in every test. The F_1 plants were all immune, like their immune parents Argentine selection, Ottawa 770B, and Bombay. The susceptible parents inoculated at the same time were invariably infected. Immunity from rust in these crosses evidently is completely dominant over susceptibility.

SEGREGATION IN THE F_2

The F_2 plants were classified into two categories with respect to rust reaction, namely, immune and susceptible. Those showing no trace of rust were placed in the immune class, while those that developed rust pustules were classed as susceptible.

[4] Thanks are due to the Division of Soils, University of Minnesota, for providing the land at Coon Creek, and to the Division of Agricultural Engineering, University of Minnesota, for that at Clearspring, for these experiments.

The results of inoculating the F_2 plants of the Argentine selection × Saginaw cross and its reciprocal are given in table 1.

TABLE 1.—*Rust reaction of the F_2 of the Argentine selection × Saginaw and Saginaw × Argentine selection crosses*

Cross	Place tested	Number of plants observed		Number of plants expected[a]		Deviation / P. E.
		Immune	Susceptible	Immune	Susceptible	
A × S	Greenhouse	209	17	211.875	14.125	1.17
A × S	Field	319	19	316.875	21.125	0.71
S × A	Greenhouse	107	9	108.750	7.250	0.99
Total	Greenhouse and field	635	45	637.500	42.500	0.59

[a] Assuming a segregation of 15 immune plants to 1 susceptible plant.

Assuming a 15:1 segregation, the deviation of the observed from the expected is in no case greater than might occur from chance alone. It will be noted that the results were the same when Argentine selection was used as the male parent as when employed as the female parent.

In those crosses in which Ottawa 770B was used as the immune parent, notes were taken on the flower color of the F_2 plants as well as on their rust reaction, since Ottawa 770B has white flowers while the susceptible parents have blue flowers. The F_2 results of the Saginaw × Ottawa 770B are shown in table 2. It will be noted that the observed numbers fit a dihybrid ratio very well. The inheritance of rust reaction in this cross is evidently simpler than in the previous cross. Immunity from rust here is

TABLE 2.—*Segregation for rust reaction and flower color of the F_2 of the Saginaw × Ottawa 770B cross*

Cross	Number of blue-blossom plants		Number of white-blossom plants	
	Immune	Susceptible	Immune	Susceptible
Saginaw × Ottawa 770B observed	441	145	142	40
" " " expected 9:3:3:1	432	144	144	48

$\chi^2 = 1.556$ $\quad p = 0.6741$

apparently determined by a single factor, as is also flower color. Rust reaction and flower color in this cross evidently are inherited independently

of each other. All white-flower segregates invariably bore yellow seed and crimpled petals, like Ottawa 770B. There is a deficiency in numbers of white plants. As might be expected, this is more pronounced when the numbers are based on the F_3 families. Results for the Winona × Ottawa 770B cross are given in table 3. Tammes (7) obtained comparable results in studying the inheritance of flower color in similar crosses. She attributes her results to a factor C^1, "this factor having a semi-lethal effect in the absence of B_1, which renders the combination of gametes white with white less viable. In consequence of this there arise capsules as compared with other genotypes averaging fewer seeds, whilst the seed formed has less germinative power."

TABLE 3.—*Segregation for rust reaction and flower color in the F_2 of the Winona × Ottawa 770B cross, based on the behavior of the F_3 families*

Cross	Number of blue-blossom plants		Number of white-blossom plants	
	Immune	Susceptible	Immune	Susceptible
Winona × Ottawa 770B observed	182	53	36	11
" " " expected 9:3:3:1	158.625	52.875	52.875	17.625

$\chi^2 = 11.32$ $p = 0.0112$

In the above table all families showing segregation for rust reaction were considered to have come from heterozygous F_2 plants and are therefore classed in the immune phenotype of that generation. All families showing segregation for flower color are classed as blue for a similar reason. Due largely to the deficiency of white plants, the deviation of the observed ratio from the theoretical is of the magnitude shown. Such a deviation could be attributed to chance only about once in a hundred times. The segregation for rust reaction alone is in good agreement with an expected 3 : 1 ratio, there being 218 plants classified as immune and 64 as susceptible, a deviation from the expected of 1.33 times the probable error. The immune variety Bombay was crossed with the susceptible varieties Saginaw, Winona, and Blue Blossom Dutch. The results of the Saginaw crosses are not reported here, as the numbers were too small to be conclusive, though the segregation appeared to be similar to that of the other crosses. The segregation for rust reaction in the F_2 of these crosses is shown in table 4.

The two susceptible parents in these crosses are very similar. They were both heavily infected with rust, as were also the susceptible segregates of the F_2. The latter stood out very sharply from the immune plants and could readily be distinguished at a distance by the abundance of rust on

TABLE 4.—*Rust reaction of the F_2 of Winona × Bombay and Blue Blossom Dutch × Bombay*

Cross	Number of plants observed		Number of plants expected		Deviation / P. E.
	Immune	Susceptible	Immune	Susceptible	
Winona × Bombay	474	153	470.25	156.75	0.51
Blue Blossom Dutch × Bombay	251	92	257.25	85.75	1.15

them. The Winona plants and the susceptible segregates of the Winona × Bombay cross were most heavily rusted, so severely, in fact, that a considerable number of the susceptible segregates were killed before they had formed seed. The ratios of the immune to susceptible plants in both crosses are in close agreement with a 3 : 1 expectation.

Back Cross. The F_1 of the Saginaw × Argentine cross was back-crossed on Saginaw, the susceptible parent. These crosses were made in the greenhouse and the seedlings from the crossed seed were inoculated with rust three times. At the same time F_2 seedlings, derived from seed of these same F_1 plants used in the back crosses, were inoculated in a similar manner and under the same conditions. The results are shown in table 5.

TABLE 5.—*Rust reaction of Saginaw × (Saginaw × Argentine) selection seedlings compared with that of F_2 seedlings of the Saginaw × Argentine selection cross*

Cross	Number of plants observed		Number of plants expected		Deviation / P. E.
	Immune	Susceptible	Immune	Susceptible	
S × (S × A)	51	18	51.75(3)	17.25(1)	0.31
S × A	107	9	108.75(15)	7.25(1)	0.99

It will be seen that the segregation of the back-cross seedlings for rust reaction fits a 3 : 1 expectation very well, while the segregation of the F_2 seedlings under the same conditions is in good agreement with a 15 : 1 expectation. These results therefore support the hypothesis that there are two duplicate factors determining rust reaction in this cross.

RUST REACTION OF THE F_3

F_3 families of the Argentine selection × Saginaw cross were tested in the field. Assuming that rust reaction in this cross is determined by two duplicate factors, a ratio of 7 : 8 : 1 would be expected in the F_3. Seven out of 16 families should breed true for immunity from rust, 8 families should segregate, 4 in a ratio of 3 immune plants to 1 susceptible, and 4 in

a ratio of 15 immune plants to 1 susceptible plant, and 1 family out of 16 should breed true for susceptibility to rust. Among the segregating families observed there were some which were clearly segregating in a 3:1 ratio and others which were clearly segregating in a 15:1 ratio. Several of these families contained between 50 and 100 plants. There were other families containing fewer plants in which the ratio of immune to susceptible plants was such that they could not be definitely assigned to either the 3:1 or the 15:1 group. Consequently, in table 6 all of the segregating families

TABLE 6.—*Rust reaction of F_3 families of the Argentine selection × Saginaw cross*

	Number of families breeding true for immunity	Number of segregating families	Number of families breeding true for susceptibility
Observed	98	88	13
Expected (7:8:1)	87.1	99.5	12.4

$x^2 = 2.73$ $p = 0.2622$

are placed in one class. The chances that such a deviation from the expected as is shown in table 6 is merely due to random sampling are about one in four. The immune class is larger and the segregating class smaller than expected. It is quite probable that several of the families placed in the immune class should go in the segregating class. For instance, in a small family segregating in a ratio of 15 immune to 1 susceptible the susceptible plants might be overlooked, as the infections were often confined in this cross to a few pustules per plant.

The F_3 families of the other crosses studied were grown at University Farm, St. Paul, the following year (1926). In order to save time and expense, an effort was made to obtain an epiphytotic of flax rust there, but the season was very unfavorable for rust development. When by midsummer it was realized that it would not be possible to obtain a uniform infection, as many as possible of the F_3 families were resown with reserve seed on a peat bog at Clearspring, Minnesota. The plants were too late to form seed, but a fairly good epiphytotic of rust was obtained.

F_3 families of two of the Ottawa 770B crosses were grown, namely, Saginaw × Ottawa 770B and Winona × Ottawa 770B. The infection on the former was not very heavy, and, consequently, the results are not sufficiently reliable to report. As has been previously mentioned, Saginaw, the susceptible parent in this cross, often develops only a few pustules of rust, and, if the plants are not subjected to a very heavy and uniform epiphytotic, some of them may be classed as immune. Under the conditions of the test, only about 90 per cent of the Saginaw plants became rusted. In the

case of the much more susceptible variety Winona, however, 100 per cent of the plants developed rust. The results of the Winona × Ottawa 770B cross are shown in table 7. In a few of the families counted as susceptible,

TABLE 7.—*Rust reaction of F_3 families of the Winona × Ottawa 770B cross*

Cross	Number of families			Total
	Immune	Segregating	Susceptible	
Winona × Ottawa 770B observed	80	152	66	298
" × " " expected	74.5	149	74.5	298

$x^2 = 1.44$ $p = 0.50$

there were a few plants which did not become rusted. It is probable that some of these were natural hybrids with immune plants. Immunity being dominant, they would, of course, be free from rust. None were counted susceptible, however, which did not show more than 50 per cent of the plants rusted. There would be one chance in every two of a deviation, as great as that shown, of the observed from the theoretical being due to random sampling.

In the Winona × Bombay cross, the observed F_3 ratio, as shown in table 8, departs considerably from the theoretical. There is only about one chance in 100 that such a departure from the expected is attributable to chance. Evidently, a disturbing influence has affected this ratio. The susceptible class is only about half as large as it should be. As was pointed out in connection with the F_2, a considerable number of the susceptible segregates were so severely rusted that they failed to form seed. This largely accounts for the small susceptible class of the F_3. In the Blue Blossom

TABLE 8.—*Rust reaction of F_3 families of the crosses Winona × Bombay and Blue Blossom Dutch × Bombay*

Cross	Number of families			Total	x^2	p
	Immune	Segregating	Susceptible			
Winona × Bombay observed	30	67	14	111		
Winona × Bombay expected 1:2:1	27.75	55.50	27.75	111	9.37	0.01
Blue Blossom Dutch × Bombay observed	34	60	35	129		
Blue Blossom Dutch × Bombay expected 1:2:1	32.25	64.50	32.25	129	0.63	0.8

TABLE 9.—*Ratios of immune to susceptible plants in 25 segregating families in each of the 3 crosses, Winona × Ottawa 770B, Winona × Bombay, and Blue Blossom Dutch × Bombay*

No. of family	Winona × Ottawa 770B		No. of family	Winona × Bombay		No. of family	Blue Blossom Dutch × Bombay	
	No. of immune plants	No. of susceptible plants		No. of immune plants	No. of susceptible plants		No. of immune plants	No. of susceptible plants
1	19	9	200	20	8	160	13	4
3	25	8	201	33	9	161	19	9
4	23	8	203	22	6	163	21	5
5	17	1	205	22	6	172	16	11
8	25	7	208	19	14	177	11	8
12	21	2	209	25	11	179	9	4
13	23	2	210	20	15	180	13	4
14	12	5	214	16	7	183	2	2
16	14	3	215	25	7	185	15	11
17	10	1	218	15	3	187	21	3
18	12	2	219	13	2	189	9	4
19	7	1	220	22	6	192	21	3
22	12	4	223	20	5	193	19	11
23	7	2	224	22	2	198	20	3
24	11	5	225	15	11	199	23	4
25	24	4	226	15	13	214	8	6
26	24	10	228	21	7	215	17	9
27	22	17	229	20	3	216	15	4
29	31	6	230	17	3	217	21	5
33	5	2	233	21	9	218	5	2
36	20	8	236	19	5	219	3	1
37	11	9	237	18	7	221	6	4
40	24	7	240	24	12	223	19	7
45	21	16	241	9	8	224	20	11
46	14	2	243	22	7	225	21	7
Totals (obs.)	434	141		495	186		367	142
Expected 3: 1	431.25	143.75		510.75	170.25		381.75	127.25
Deviation / P. E.	0.39			2.07			2.24	

Dutch × Bombay cross, x^2 is less than one and, consequently, p indicates a close agreement between the observed and theoretical ratios.

Assuming that there is but a single-factor difference between immunity and susceptibility in the Ottawa 770B and Bombay crosses and a complete dominance of immunity over susceptibility, the segregating families of the F_3 should give 3 (immune) : 1 (susceptible) ratios. The observed ratios fulfill this expectation, as is shown in table 9. The deviation from the expected is in no case greater than three times the probable error.

BREEDING WORK

The parents used in the crosses discussed possess, as has been pointed out, many valuable characteristics, included among them immunity from rust and a high degree of wilt resistance, though each of them lacks one or more desired characters.

As the primary object of the investigations is to develop varieties of seed and fiber flax possessing not only immunity from rust but wilt resistance and other desirable characters as well, numerous selections have been made with these points in mind.

The results of the inheritance studies have furnished a valuable guide for the selection work. By selecting from F_3 families breeding true for immunity from rust and flower color, one can be assured that the progenies of these selections will also breed true. However, because of the occurrence of natural crossing (5), it is advisable to protect the selected plants from foreign pollen. Selections were, of course, also made from segregating families which appeared promising. In making the original selections, particular attention was given to earliness, height, diameter of stem, size and color of seed, size and color of flowers, degree of branching, and yield of seed, as well as to immunity from rust. Types unsuitable for fiber purposes might well prove valuable for seed purposes. The plan for the further testing of the selections calls for a wilt-resistant test on soil infested with the wilt organism, finally a yield and oil test on those surviving selections suitable for seed flaxes, and a yield and quality test especially of the stems and their fiber content of the surviving tall selections suitable for fiber flaxes.

It was evident from a study of the F_3 families of the Ottawa 770B and Bombay crosses and of the F_3 and F_4 families of the Argentine selection crosses that immunity from rust could be combined with many desirable qualities not possessed by the immune parents. Thus, in figure 1, A, (right) we see it combined with greater height in an F_3 family of the Saginaw × Ottawa 770B cross. This family bred true for immunity from rust and for white flowers and yellow seed. The immune parent, Ottawa 770B, is shown at the left. The white-flower selections from the Ottawa

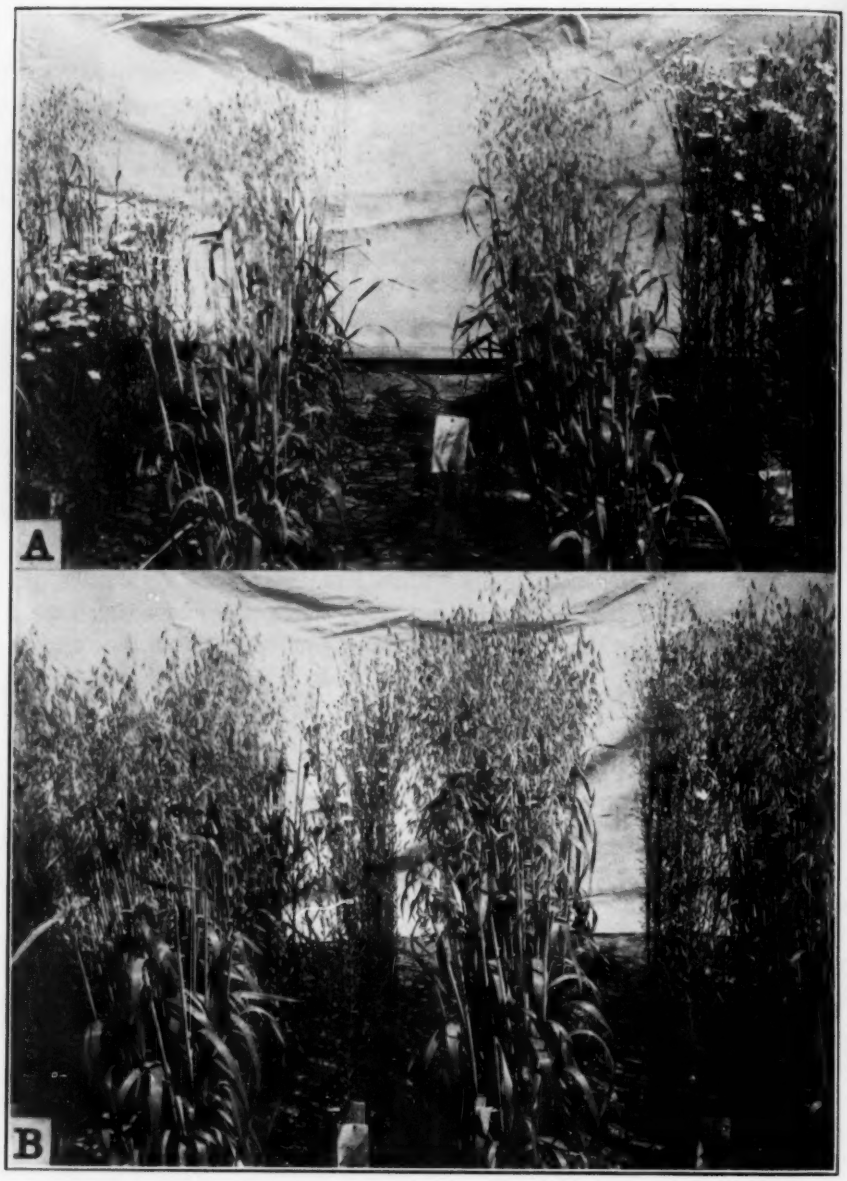

FIG. 1. A. Left; Ottawa 770B, one of the rust-immune parents. Right; Tall, white-flower F_2 family of the cross Saginaw × Ottawa 770B. This family bred true for immunity from rust, flower, and seed color. B. Two tall blue-flower F_2 families of the Saginaw × Ottawa 770B cross.

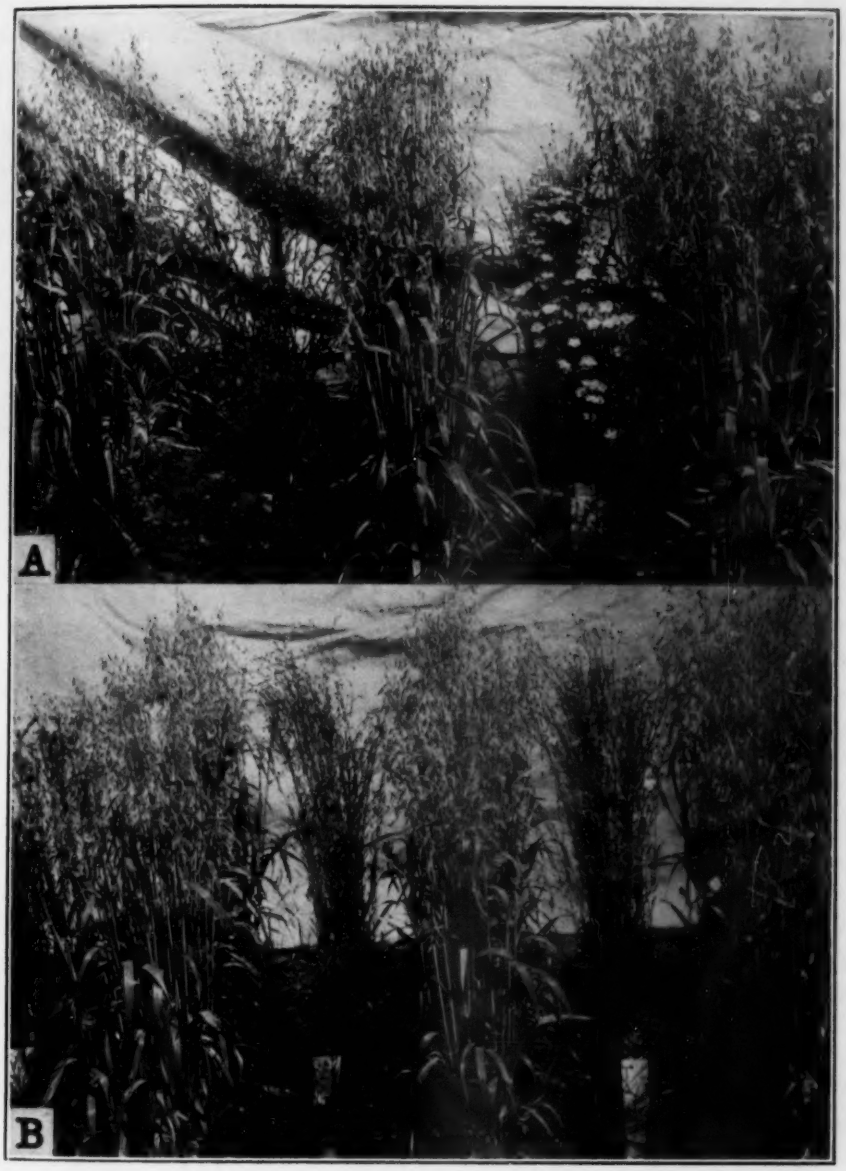

FIG. 2. A. Left; Saginaw fiber flax, a susceptible parent. Right; Argentine selection, immune from rust and resistant to wilt. B. Two tall F_4 families of the Saginaw × Argentine selection cross. These resemble the susceptible parent in height and earliness and the immune parent in freedom from rust.

770B selections, because of low yield, may not be suitable for seed flaxes but may be valuable as fiber flaxes provided suitable yield and quality of fiber can be obtained in them. Their distinctive flowers would be useful in keeping them pure. In figure 1, B, are shown two of the taller blue-flower F_3 families from this same cross. Some of these bred true for immunity from rust and for blue flowers and brown seed; moreover, they were much taller and earlier than Ottawa 770B, the immune parent. Promising selections of the seed flax type were obtained from the Winona × Ottawa 770B cross as well as from the Argentine selection, and a few from the Bombay crosses. Figure 2 shows the great difference in earliness between Saginaw and Argentine selection, while figure 2, B, shows F_4 selections from a cross between these two varieties which have the height and earliness of the susceptible parent and the freedom from rust of the immune parent.

Although the writer did not have an opportunity to test the relative wilt resistance of many of the selections, it is very probable that a number of them will prove wilt-resistant as well as immune from rust. The parents Argentine selection and Winona both possess wilt resistance to a high degree, and the fact that the former also is immune from rust indicates that the two qualities can be combined in the one variety. The development of improved varieties of seed and fiber flax combining resistance to these two major diseases will constitute an important step. Further research should aim to introduce resistance to still other diseases, such as pasmo, browning, and anthracnose, into new varieties.

SUMMARY

1. Argentine selection, Ottawa 770B, and Bombay, three varieties immune from flax rust, were used as parents in the investigations reported.

2. All three varieties have been inoculated by the writer with several North American collections of flax rust and with one European collection. The first two varieties also have been tested by Hiratsuka in Japan. They have remained immune in all tests.

3. The immune varieties were crossed with susceptible varieties of fiber and seed flax. The principal susceptible parents used were Saginaw and Winona. The latter is more susceptible than the former, particularly in the later stages.

4. Immunity from rust proved completely dominant in all crosses. The F_1 plants were invariably immune.

5. Evidence was obtained in the case of the Argentine selection × Saginaw cross which indicates that immunity from rust depends on two duplicate factors for its expression.

6. In the crosses in which Ottawa 770B and Bombay were used as immune parents, the results of inoculations indicate but a single factor difference between immunity and susceptibility.

7. Immunity from rust was apparently inherited independently of flower and seed color in crosses of Ottawa 770B on Winona and Saginaw.

8. Numerous selections were made from F_3 and F_4 families of the various crosses. Many of these appeared promising, some for fiber purposes and others for seed purposes.

9. Immunity from rust was combined in these selections with various characters not possessed by the immune parents, such as greater height, greater earliness, fewer branches, and difference in color and size of flowers and seeds.

10. Improved selections combining immunity from rust and wilt resistance should also be obtainable from the crosses made.

LITERATURE CITED

1. BARKER, H. D. A study of wilt resistance in flax. Minn. Agr. Exp. Sta. Tech. Bul. 20. 1923.
2. DAVIN, ADELAIDE G., and G. O. SEARLE. A botanical study of the flax plant.: IV the inheritance and inter-relationship of the principal plant characters. Jour. Tex. Inst. **16**: T61–T83. 1925.
3. HENRY, A. W. Flax rust and its control. Minn. Agr. Exp. Sta. Tech. Bul. 36. 1926.
4. ————. Inheritance of immunity from *Melampsora lini*. Phytopath. **16**: 87. 1926.
5. ————, and C. TU. Natural crossing in flax. Jour. Amer. Soc. Agron. **20**: 1183–1192. 1928.
6. HIRATSUKA, N. Studies on the flax rust. Trans. Sapporo Nat. Hist. Soc. **10**: 1–27. 1928.
7. TAMMES, TINE. The genetics of the genus *Linum*. Bibliographia Genetica **4**: 1–36. 1928.
8. TISDALE, W. H. Flax wilt: A study of the nature and inheritance of wilt resistance. Jour. Agr. Res. **11**: 573–605. 1917.

GROWTH OF PLANT PATHOGENIC BACTERIA IN SYNTHETIC CULTURE MEDIA WITH SPECIAL REFERENCE TO PHYTOMONAS MALVACEARA

I. M. Lewis

INTRODUCTION

Although synthetic culture media have been extensively employed in studies of bacterial nutrition and for the purpose of differentiating species or strains in other groups, there appears to have been but few similar studies made on the species pathogenic to plants. Following the lead of Erwin F. Smith nearly all investigators have made use of the synthetic media of Uschinsky (8), Fermi (8), and Cohn (8) in the study and description of new species.

It is obvious that such a complex medium as that of Uschinsky is not well suited to the purpose of determining precisely which carbon or nitrogen compounds are suitable for the growth of an organism. Ability to grow in either Fermi's or Cohn's solution denotes utilization of ammonia as a source of nitrogen, but failure to grow does not definitely prove inability to assimilate ammonia, since either glycerin or tartaric acid may be unsuited as a source of carbon. The accurate determination of nutritive requirements is possible only when carbon and nitrogen compounds are so combined as to afford but a single source of each in the culture medium. When a variety of such media is employed it is possible definitely to determine carbon and nitrogen requirements.

Use of such media has often been made in the study of other groups, and our knowledge of bacterial nutrition has been markedly advanced by such studies. In addition valuable means of differentiating closely related species or strains based on utilization of a single source of nitrogen or carbon have been discovered. Koser (5, 6) has shown that differentiation within the colon group may be accomplished by employing synthetic media containing citrate as the sole source of carbon or uric acid as the source of nitrogen. Similarly Simmons (7) and others have shown differentiation within the paratyphoid group. Recently, Frazier and Rupp (3) have suggested the possibility of grouping proteolytic bacteria by growth in media containing ammonia or urea with the addition of sugars or organic acids.

Braun and his collaborators have emphasized the necessity of certain refinements in technique not generally practiced by earlier investigators. Braun and Cann-Bronner (1) were apparently the first to recognize the importance of testing the ability of an organism to grow in serial trans-

TABLE 1.—*The growth of plant-pathogenic bacteria in the synthetic media of Uschinsky, Fermi, and Cohn. Compiled from data published by various authors*

Name of species	Medium			Author
	Uschinsky	Fermi	Cohn	
Erwinia amylovora	–[c]		–	Smith
" aroidea	+–[b]			Townsend
" atroseptica	+[a]	+	–	Morse
" carotovora	+			Jones
" nicotiana	+–		–	Smith
" oleracea	+	+		Harrison
" solanisapra	+	+		Harrison
" tracheiphila	+–	+–	+–	Smith
Phytomonas alboprecipitans	+	+	–	Rosen
" angulata	+			Fromme and Murray
" apii	–		–	Jagger
" aptata	+	+	–	Brown and Jamison
" atrofaciens	+	+	–	McCulloch
" beticola	+	+	–	Brown
" bowlesia	+	+	+–	Lewis and Watson
" campestris	+–	+–	+–	Smith
" cannae	+	+	+–	Bryan
" citrarifaciens	+	+	+	Lee
" coronafaciens	+	+	+	Elliott
" cucurbitae	+		–	Bryan
" delphinii	+	+	+	Bryan
" dissolvens	+–	–	–	Jones
" erodii	+	+	+–	Lewis
" exitosa	–	–	–	Gardner and Kendrick
" flaccumfaciens	+	+–	+–	Hedges
" gummisudans	+–	+–	+–	McCulloch
" holci	+	+	–	Kendrick
" hyacinthi	+			Smith
" insidiosum	–	–	–	Jones and McCulloch
" lachrymans	+	+	+	Smith and Bryan
" maculicola	+–		+–	Smith
" malvaceara	+		–	Smith
" marginale	+	–	–	Brown
" marginata	+	+	+	McCulloch
" martyniae	+	+	+	Elliott
" mellea	+	+		Johnson
" michiganensis	–	–	–	Smith
" mori	+		–	Smith
" panici	+	+	+	Elliott
" papulans	+		–	Rose
" pelargoni	+	+	–	Brown
" phaseoli	+–			Smith
" pruni	+			Brown
" puerariae	+	+		Hedges
" rathayi			–	Smith
" savastonoi	+	+	–	Smith
" solanaceara		+–	–	Smith
" stewarti	+	+–	–	Smith
" syringae	+	+	+–	Bryan
" translucens	+	–	–	Jones *et al.*
" trifoliora	+	+	–	Jones *et al.*
" tumefaciens	+–		–	Smith and Townsend
" vasculara	+–		–	Smith
" vignae	+	+	–	Gardner and Kendrick
" viridilivida	+	+	+–	Brown
" viridifaciens	+	+	–	Tisdale and Williamson
" vitians	+	–	–	Brown
" xanthochlora	+		–	Smith

[a] + = "Good growth." [b] +– = "Feeble," "scant," "wanting." [c] – = No growth.

plants in simple synthetic media. Their results show that in some cases an organism may be able to grow when transplanted from complex media but fail to maintain growth in successive transplants in the synthetic medium. This appears to be true particularly in the case of old cultures grown on such media as beef-extract-peptone broth or agar. Since an organism should be able to maintain itself indefinitely in suitable media when transferred at proper intervals it is only necessary to make sufficient serial transplants in order to eliminate this source of error. Braun and Cahn-Bronner (1) consider growth in less than three serial transplants as unreliable. They called attention also to the necessity of affording proper aeration for cultures in such simple media as must be employed. They made use of small flasks containing only sufficient medium to form a shallow layer. Positive results often were obtained by this method when no growth occurred in tubes containing deep layers.

Before beginning the experimental work of the present investigation, it was deemed essential to review the literature of the group thoroughly to determine the present status of our knowledge of the nitrogen and carbon sources utilized by the various species. In so far as it was possible to do so, original descriptions of the species have been consulted. Since data are limited principally to the tests made in the media of Uschinsky, Fermi, and Cohn it seems desirable to tabulate all of the results for convenient reference. (Table 1.) The species names published in the third edition of Bergey's Manual of Determinative Bacteriology have been adopted.

In considering table 1 it must be borne in mind that little or nothing is said as to method of inoculation, time required for growth, number of strains tested, aeration of cultures, or ability to grow in serial transplants. Such expressions as "growth scant," "slight," "feeble," "transient," "doubtful," or "wanting" are frequently met.

Table 1 shows that most of the species are capable of growth in Uschinsky's medium, thus denoting ability to use ammonia or asparagin, or both, as the source of nitrogen, and one or more of the compounds, asparagin, glycerin, or lactate, as the source of carbon. A comparison of the results obtained in the media of Fermi and Cohn shows that Cohn's medium is less satisfactory. Since these media are essentially alike except for the carbon compound, it appears that tartrate is less suitable than glycerin for most species.

More precise data are available for a few species only. Jones (4) tested the growth of *Erwinia carotovora* in a synthetic medium containing ammonia as a sole source of nitrogen and sodium acetate or formate as the only source of carbon. The organism assimilates ammonia and acetate but not formate. Smith (9, p. 290) obtained no growth of *E. tracheiphila* in

asparagin water containing dextrose and mineral salts or in filtered boiled river water containing 1.0 per cent sodium asparaginate, 1.0 per cent dextrose, and 2.0 per cent glycerin. Similar results were obtained when ammonium lactate or tartrate was substituted for sodium asparaginate. Bryan (2) tested the growth of *Phytomonas delphinii* in solutions of peptone, asparagin, and the ammonium salts of citric, tartaric, and succinic acids. The organism uses ammonia, citric, and succinic acids but not tartaric acid. She also reported growth in Uschinsky's and Fermi's but not in Cohn's medium. Smith (10) studied sixteen strains of *Ph. solanaceara*. He found that both urea and ammonia were suitable sources of nitrogen and that growth occurred with dextrose, sucrose, and sodium salts of citric, malic, tartaric, and succinic acids. There was no growth with lactate, acetate, or butyrate. Higgins found that a species pathogenic to peppers was unable to grow in a medium containing only asparagin. The most complete study of the carbon and nitrogen requirements of a plant-pathogenic organism appears to be that of Honing for *Ph. solanaceara*. The results of this study are fully summarized by Smith (10, pp. 244–254). It is shown that asparagin, glycocoll, potassium nitrate, potassium nitrite, and ammonia may serve as sources of nitrogen in the presence of a suitable source of carbon. Certain carbon compounds were found to be absolutely nonusable in the combinations tested. These included glycogen, starch, lichenin, sodium acetate, and sodium butyrate. The most notable feature of this study was the extreme variability of the several strains in the same medium. Some strains almost invariably failed to grow in media which supported growth of others. Similar results were obtained by Braun (1) and by Koser (5) in other groups, thus emphasizing the necessity of including several strains of a species in studies of this kind.

From the data at hand it is obvious that our knowledge concerning utilization of either carbon or nitrogen compounds by species of plant pathogens is far from complete. It appears that ammonia is suitable for the growth of most species and that variation is to be expected in the utilization of various sources of carbon. Before such tests can be considered as of differential value among closely related species, it must be shown that uniformity prevails among several strains of such species. That such tests might prove to be of such differential value, as has already been shown for other groups, seems probable.

The present investigation was originally planned to include strains of more than one species from various sources. This was found impracticable because of the difficulty of obtaining sufficient numbers of strains. It was then decided to limit the investigation to one species. Because of its wide distribution, the certainty of identification, and availability of material for

isolation of numerous strains from different localities, *Ph. malvaceara* was chosen as the most desirable species available. All experiments were performed in the Laboratory of Plant Pathology at the University of Texas.

SOURCE OF TEST STRAINS

The strains used throughout the study were freshly isolated from young cotton plants growing in different fields in the vicinity of Austin, Texas. The organism is readily obtained from the young, water-soaked, angular spots of leaves. In order to minimize possibilities of obtaining closely related strains one strain only was isolated from each plant. The cultures were replated and fished a second time from well-separated single colonies before beginning the experiment. All of the cultures were typical of the organism described by Smith (11, pp. 314–339). Briefly, the salient features are as follows: Nitrates not reduced; starch hydrolyzed; gelatin liquefied slowly; litmus-milk alkaline with complete coagulation followed by slow peptonization; growth on potato light yellow, very copious, the water at the base becoming completely solid; growth occurs in Uschinsky's and Fermi's but not in Cohn's medium. Each strain was tested for pathogenicity by spraying water suspensions on young cotton plants growing in flower pots. The production of numerous typical water-soaked angular spots occurred after about two weeks. A total of 14 such strains was obtained.

METHODS OF CULTIVATION

All glassware used in the experiments was treated with acid-cleaning fluid and then thoroughly rinsed with tap and distilled water. The water used for preparation of media was redistilled from the stock supply. The chemicals employed were of the highest purity obtainable and further purification was not attempted.

Since the object of the investigation was to determine organic rather than inorganic requirements for growth, experiments were not performed to determine the most favorable mineral solution. The basic solution adopted was prepared according to the recent formula of Frazier and Rupp (3). This solution contains dibasic potassium phosphate 0.31 per cent, monobasic potassium phosphate 0.08 per cent, potassium chloride 0.02 per cent, and magnesium sulphate 0.02 per cent. In media containing ammonia as the only source of nitrogen, 0.25 per cent of sodium ammonium phosphate was substituted for the potassium phosphates.

The various carbon and nitrogen compounds were dissolved in these constant mineral solutions and were then distributed in 5 to 8 cc. quantities in large test tubes. The sugar-containing media were discontinuously sterilized at 100° C. Media containing urea were sterilized by filtration

and then aseptically tubed. All other media were sterilized in an autoclave at 12 to 15 pounds pressure. Reaction of the finished media varied from about pH 7.6 to pH 6.8. *Phytomonas malvaceara* grows very well in beef-extract-peptone broth at these reactions.

The sodium salts of the various organic acids were supplied in 0.2 per cent, the carbohydrates and alcohols in 0.5 per cent concentration. The nitrogen-bearing compounds were added as follows: Asparagin, glycerin, aspartic acid, glutamic acid, alanine, edestine, 0.3 per cent, urea 0.1 per cent, and tyrosine 0.1 per cent.

The inoculum was prepared by rubbing up a small bit of the growth from a 48-hour-agar-slant culture in about 3 cc. of distilled water. One loopful of the suspension was used for inoculating each tube of synthetic medium. This amount was sufficient to cause growth in control tubes of plain beef-extract-peptone broth. During the period of incubation the test-tube cultures were laid on a sloped surface to afford better aeration. Results were recorded as negative if visible growth had not occurred at the end of 30 days.

Following Braun's method of serial cultivation, transplants were made from all positive tubes as soon as the growth had apparently reached its maximum. The time varied somewhat with different strains and quite markedly for different media. Results were not recorded as positive until growth occurred in the fourth successive transplant. Agar plate cultures were prepared from the final positive tubes in order to make certain that contaminations had not been picked up in the series.

RESULTS

Results of the experiments to determine utilization of carbon compounds with ammonia or asparagin are shown in table 2. All strains behaved uniformly in all of the combinations tested.

It is seen that either ammonia or asparagin is a suitable source of nitrogen in the presence of a satisfactory source of carbon but that asparagin fails to supply both nitrogen and carbon. Growth was very copious in the case of glucose, sucrose, lactose, galactose, and levulose; less abundant but vigorous with maltose, starch, glycogen, inulin, raffinose, glycerin, and the sodium salts of acetic, citric, butyric, lactic, and succinic acids. No growth whatever occurred with arabinose, mannitol, dulcitol, salicin, formate, tartrate, or oxalate after the first transplant and there never was more than a scant, almost invisible, clouding of the medium in any of the first tubes. In all suitable media growth was as prompt and vigorous in the last set of tubes as in the original. The results would not have been changed in any way by eliminating the third and fourth serial transplants. The additional

TABLE 2.—*The growth of Phytomonas malvaceara in synthetic media containing ammonia or asparagin and various sources of carbon*

Sources of carbon	Sources of nitrogen		
	Ammonia	Asparagin	None
Asparagin		−	
Glucose	+	+	−
Levulose	+	+	−
Sucrose	+	+	−
Maltose	+	+	−
Lactose	+	+	−
Galactose	+	+	−
Arabinose	−	−	−
Raffinose	+	−	−
Xylose	+	−	−
Starch	+	−	−
Inulin	+	−	−
Glycogen	+	−	−
Glycerol	+	+	−
Mannitol	−	−	−
Dulcitol	−	−	−
Salicin	−	−	−
Sodium acetate	+	−	−
" citrate	−	+	−
" butyrate	+	+	−
" formate	−	−	−
" lactate	+	+	−
" tartrate	−	−	−
" succinate	+	+	−
" oxalate	−	−	−

nitrogen-bearing compounds were tested to determine ability to obtain both nitrogen and carbon from the same compound or nitrogen in the presence of a suitable source of carbon. The results of these tests are shown in table 3.

The fourteen strains behaved uniformly. Growth occurred with all the nitrogen-bearing compounds tested when a suitable source of carbon was present. Four only of the compounds, peptone, glutamic acid, alanine, and edestine, were capable of supplying both carbon and nitrogen.

The work of Jones, Smith, Bryan, and Honing on other species of plant-pathogenic bacteria affords some basis for comparing the utilization of salts of organic acids in the presence of ammonia. These results are given in table 4.

It is seen that both citrate and succinate are suitable for all of the species tested. Some of the compounds appear to have differential value. These

TABLE 3.—*The growth of Phytomonas malvaceara in synthetic media containing sucrose or glycerol and various sources of nitrogen*

Source of nitrogen	Source of carbon		
	Sucrose	Glycerol	No additional carbon
Peptone	+	+	+
Ammonia	+	+	–
Asparagin	+	+	–
Urea	+	+	–
Uric acid	+	+	–
Glutamic acid	+	+	+
Aspartic acid	+	+	–
Glycine	+	+	–
Alanine	+	+	+
Tyrosine	+	+	–
Leucin	+	+	–
Cystine	+	+	–
Edestine	+	+	+
None	–	–	–

results suggest the possibility of differentiating between species by differences in utilization of nitrogen and carbon compounds.

TABLE 4.—*Utilization of salts of organic acids and ammonia by four species of plant-pathogenic bacteria*

Name of organism	Carbon compounds									Author
	Acetate	Butyrate	Citrate	Formate	Lactate	Malate	Oxalate	Succinate	Tartrate	
E. carotovora	+			–						Jones
Ph. delphinii			+					+	–	Bryan
Ph. solanaceara	–	–	+		–	+		+	+	Smith
Ph. malvaceara	+	+	+	–	+		–	+	–	Lewis
Ph. solanaceara	–		+		+			+	+	Honing

SUMMARY

It has been shown that our knowledge of carbon and nitrogen utilization by species of plant-pathogenic bacteria is incomplete, due to few investi-

gations of the subject, the use of a too limited variety of culture media, and questionable technique.

Fourteen strains of *Phytomonas malvaceara* were found uniform in regard to carbon and nitrogen compounds that support growth. The organism assimilated ammonia and all of the various more complex nitrogenous compounds tested when supplied with various sources of carbon. Peptone, alanine, glutamic acid, and edestine support growth in the absence of additional carbon. Some of the carbon compounds tested failed to support growth in the presence of ammonia or asparagin. All results are based on ability to maintain growth through four successive transplants.

THE UNIVERSITY OF TEXAS,
AUSTIN, TEXAS.

LITERATURE CITED

1. BRAUN, H., and C. E. CAHN-BRONNER. Ueber die synthetischen Fähigkeiten pathogener Bakterien und ihr biologisches Verhalten unter einfachen Ernährungsbedingungen. I. Die Nährungsbedurfnisse des Paratyphus B. Bacillus; sein Wachstum und seine Eigenschaften beim Aufbau aus einfachen chemischen Verbindungen. Centralb. Bakt. I Abt., **86**: 1–16. 1921.
2. BRYAN, MARY K. Bacterial leaf spot of Delphinium. Jour. Agr. Res. **28**: 261–270. 1924.
3. FRAZIER, W. C., and PHILIP RUPP. Studies on the proteolytic bacteria of milk. IV. Action of proteolytic milk bacteria on amino acids and other simple nitrogenous compounds. Jour. Bact. **16**: 231–245. 1928.
4. JONES, L. R. *Bacillus carotovorous*, n. sp., die Ursache einer weichen Fäulnis der Möhre. Centralb. Bakt., II. Abt., **7**: 12–21. 1901.
5. KOSER, S. A. Utilization of salts of organic acids by the *colon-aerogenes* group. Jour. Bact. **8**: 493–520. 1923.
6. ———. The employment of uric acid synthetic medium for the differentiation of *B. coli* and *B. aerogenes*. Jour. Infect. Dis. **23**: 377–379. 1918.
7. SIMMONS, J. S. A culture medium for differentiating organisms of *typhoid-colon-aerogenes* groups and for isolation of certain fungi. Jour. Infect. Diseases **39**: 209–214. 1926.
8. SMITH, ERWIN F. Bacteria in relation to plant diseases. Vol. 1. Carnegie Inst. Wash. Pub. 27. 1905.
9. ———. Bacteria in relation to plant diseases. Vol. 2. Carnegie Inst. Wash. Pub. 27. 1911.
10. ———. Bacteria in relation to plant diseases. Vol. 3. Carnegie Inst. Wash. Pub. 27. 1914.
11. ———. An introduction to bacterial diseases of plants. 688 pp. W. B. Saunders Co., Philadelphia and London. 1920.

A NEW DIPLODIA EAR ROT OF CORN

A. H. Eddins[1]

INTRODUCTION

In August, 1928, an ear of corn was collected in the experimental plots at Gainesville, Florida, rotted and blackened by a Diplodia organism that differs from *Diplodia zeae* (Schw.) Lév. and *D. macrospora* Earle in color of mycelium and color, shape, size, and septation of spores (1). The causal fungus was also found later in the season on old dead stalks of corn. The evidence now available indicates that this new ear rot is widely distributed in the State because diseased ears have been collected in Hillsborough, Clay, Gadsen, Jackson, and Okaloosa counties, Florida.

SYMPTOMS

The mycelium of the Diplodia causing this new ear rot penetrates the cob, kernels, husks, and stalk. In contrast to the white mold formed on the kernels and husks of severely diseased ears infected by *D. zeae* or *D. macrospora* (1), the causal fungus forms a dark brown, felt-like mold on these parts (Fig. 1, A and B). The interior of the severely diseased kernel consists of a mass of blackened tissue and fungous hyphae in which pycnidia often are imbedded. All or a part of the ear may be invaded, although the butt end is most often diseased. On some ears only a few scattered kernels show the characteristic blackening. On others the diseased kernels cannot be detected until they are germinated.

Stalks usually are diseased at the lower nodes and internodes. The pith is blackened and the pycnidia are imbedded either in the outer tissue of the stalks at nodes and internodes or they occur in stromata at the nodes where sufficient moisture has been retained in the cavity formed by the stalk and sheath for the growth of the fungus on the surface (Fig. 1, C). A characteristic that distinguishes this disease on the stalk from that caused by *D. zeae* and *D. macrospora* is that the pycnidia of the causal organism which are imbedded in the stalk emerge through longitudinal cracks in the epidermis (Fig. 2), while those of the other two organisms never make elongated cracks, although, when mature, they break out through the epidermis.

The disease has not been observed on the roots of plants in the field. However, the roots of seedlings grown in sterilized sand inoculated with a pure culture of the fungus were severely rotted and blackened, and the plants were either stunted in growth or killed.

[1] Grateful acknowledgment is made to Dr. G. F. Weber for suggesting the investigation of this problem and to Dr. W. B. Tisdale for aid in preparing the manuscript.

CAUSAL ORGANISM

Morphology

Pycnidia: The pycnidia occur in clumps or series and break out through longitudinal cracks in the epidermis of old stalks or occur in stromata on the surface at nodes, on the husks, and on the surface and interior of kernels. They are subglobose to globose, black in color, and measure 170–470 μ in diameter.

Pycnospores: Young pycnospores, borne on short conidiophores, are nonseptate, colorless, and granular when young, becoming brown at maturity. Mature spores are generally uniseptate, nonseptate dark colored spores occurring rarely. The dark spores frequently have longitudinal striations on their surfaces, measure 19–31 x 11–15 μ, mostly 24–28 x 13–14 μ, and average 25.3 x 13.3 μ. The spores germinate within two to four hours in tap water at room temperature. While in the process of germination

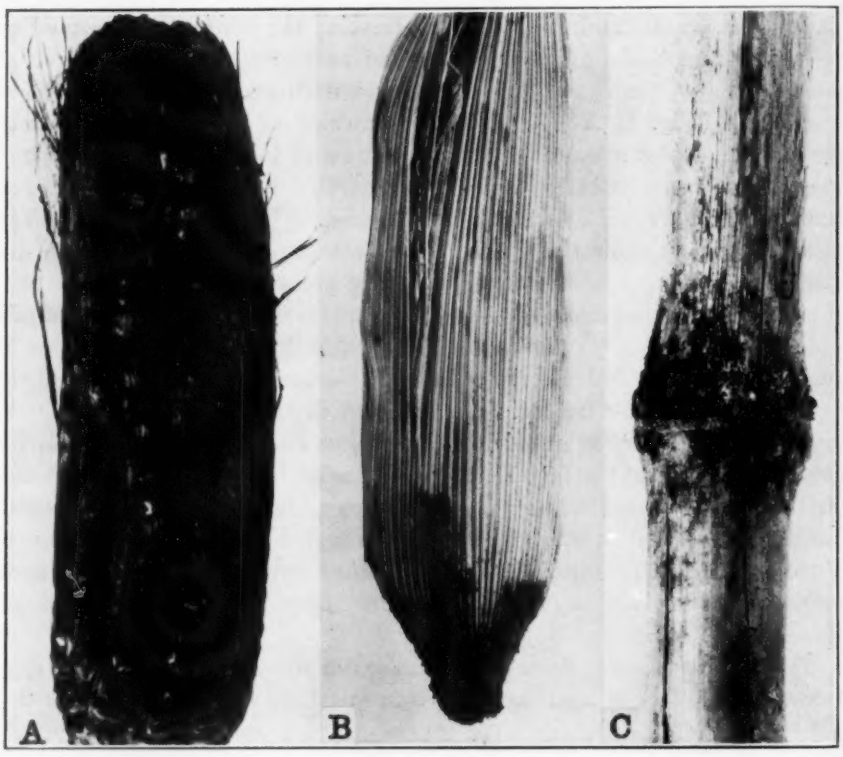

FIG. 1. A. An ear of corn severely rotted and blackened by *Diplodia frumenti*. B. Ear showing pycnidia of *D. frumenti* in stroma on the husks at the butt end. C. Pycnidia of *D. frumenti* in stroma on the surface of a stalk at the node.

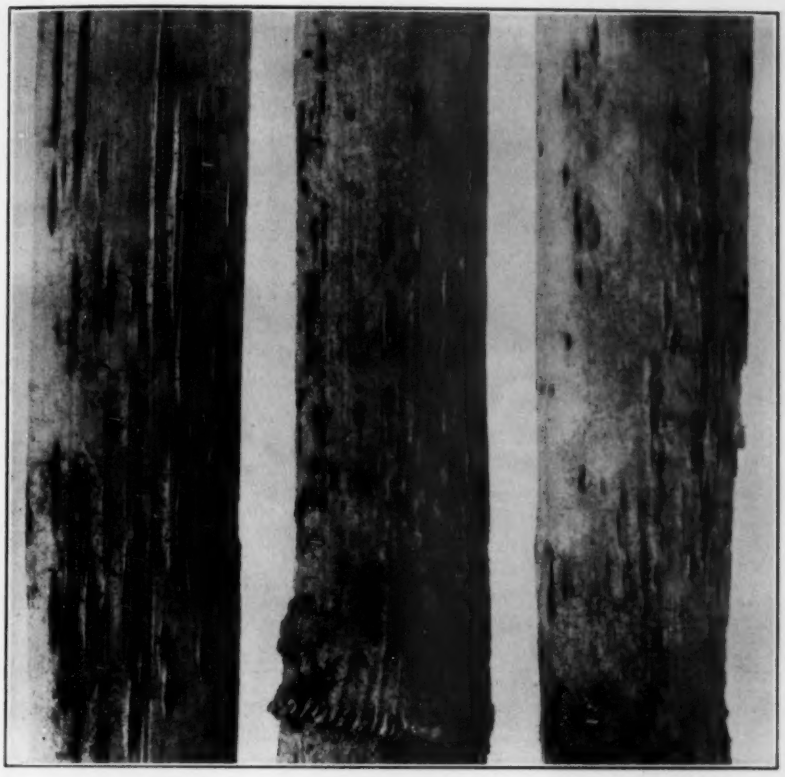

FIG. 2. Pycnidia of *Diplodia frumenti* breaking out through longitudinal cracks in the epidermis of stalks. × 2.

the spores swell at one end and the wall is slit in a ragged manner as the germ tube emerges.

Mycelium: On potato-dextrose agar, the young mycelium is hyaline and granular, septate and branched, producing a loose, flocculent, white aerial growth. As it grows older, the mycelium becomes brown and forms stromatic masses on the surface of the media. On badly diseased ears, the fungus forms brown, felty masses of mycelium on the kernels and husks.

Chlamydospores: Chlamydospores are produced in old cultures in which the nutrients have been exhausted. They are dark brown, intercalary, usually in chains.

Drawings of spores, mycelium, and chlamydospores of the fungus are shown in figure 3.

Taxonomy

In addition to *Diplodia zeae* and *D. macrospora*, one other Diplodia has been mentioned as occurring on corn. This organism was collected in 1886

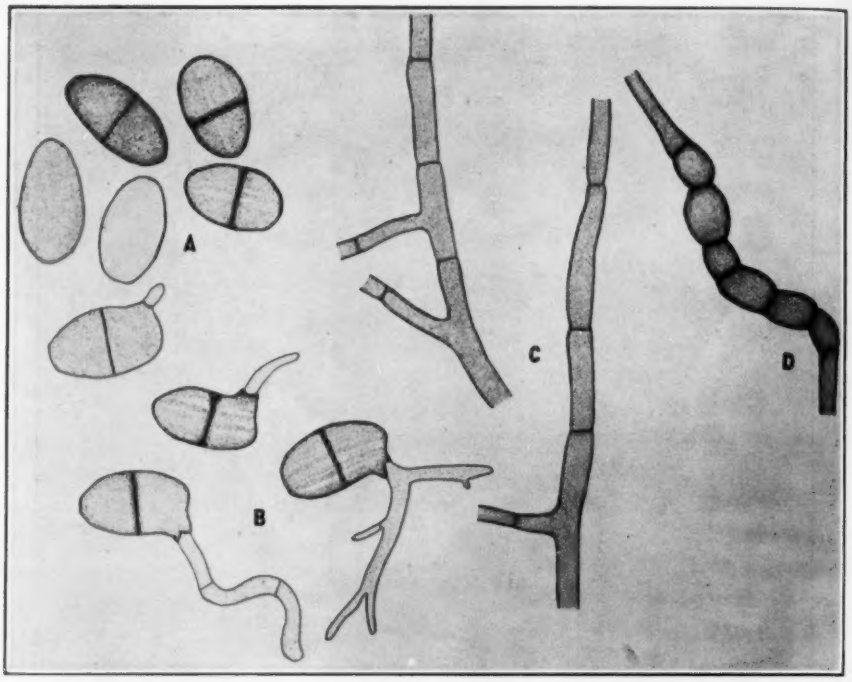

FIG. 3. Diplodia ear-rot fungus. A, various types of pycnospores; B, germinating pycnospores; C, mycelium; D, chlamydospores. ×720.

by Langlois in Louisiana on old dead stalks of corn and was described and named *D. frumenti* by Ellis and Everhart (2), whose description is as follows:

"*Diplodia frumenti*, E. and E.—On dead stalks of *Zeae mays*, June. Langlois, No. 493. Perithecia globose, sometimes with a stout, cylindrical ostiolum, subcaespitose or often seriate, bursting out through longitudinal cracks; sporules elliptical, brown, 1—septate, 15–18 x 12 μ, on stout pedicels. Very different from *Diplodia zeae*, Lév."

The Diplodia on corn in Florida agrees with the above description except that dark, nonseptate spores as well as uniseptate spores occur, and the limits in dimensions of the spores of the Florida organism exceed those of Ellis and Everhart's fungus. The spores of the Florida fungus measure 19–31 x 11–15 μ and those of *D. frumenti*, as described by Ellis and Everhart, measure 15–18 x 12 μ. The smaller size given for the latter may have been due to the possibility that only a few spores were measured and that these, if from old herbarium material, had shrunken. In obtaining the dimensions of the spores of the Florida Diplodia 200 spores from material fresh from the field were measured.

A specimen of the Diplodia on an old dead cornstalk collected from a field at Gainesville, Florida, in April, 1929, was sent to Dr. C. L. Shear, Washington, D. C., who identified it as *D. frumenti*. Pycnospores of this fungus cannot be distinguished from those of the Diplodia species which naturally occur on sweet potatoes, Citrus and cotton. They are similar in shape, septation, and color (Fig. 4) and have about the same dimensions, as is shown in table 1.

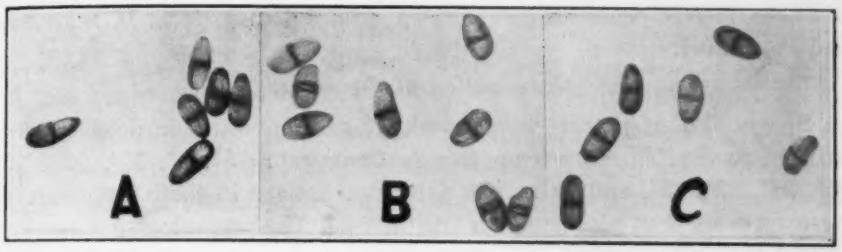

FIG. 4. Photomicrographs of pycnospores. A, *Diplodia frumenti;* B, *Physalospora rhodina;* C, *Diplodia tubericola.* ×400.

TABLE 1.—*Dimensions of 200 pycnospores each of the Diplodia species that occur naturally on corn, sweet potatoes, Citrus, and cotton when produced on ears of corn that were artificially inoculated with pure cultures of these organisms*

Source of pure cultures used for inoculation	Spore dimensions in microns	
	Limits in dimensions	Average dimensions
Corn stalks	19–31 x 11–15	25.3 x 13.3
Rotted sweet potatoes	18–31 x 9–15	24.9 x 12.5
Rotted oranges	21–30 x 10–15	27.2 x 12.6
Rotted cotton bolls	18–29 x 10–15	22.8 x 12.7

Inoculation tests have shown also that the Diplodia species on sweet potatoes, Citrus, and cotton cause a dry rot of ears of corn and the symptoms of the disease are similar to those caused by the Diplodia that naturally occurs on corn. Furthermore, the Diplodia from corn artificially inoculated into sweet potatoes, Citrus and cotton causes a rot of these hosts and the symptoms of the disease are similar to those of the diseases caused by the *Diplodia* spp. that naturally occur on these plants. Stevens (6) has shown that the common Diplodia that occurs on Citrus, *D. natalensis* Evans, and the common one on cotton, *D. gossypina* Cke., are one species, the perfect stage of which is *Physalospora rhodina* (Berk. & Curt.) Cooke. In 1911 Fawcett and Burger (3) found that Diplodia isolated from gumming peach trees caused a gumming of orange trees and Diplodia isolated

from orange trees caused a gumming of peach trees. Other workers (4, 5, 7) have also shown that *D. natalensis* and *D. gossypina* cause a rot of sweet potatoes similar to the rot caused by *D. tubericola* Ell. and Ev., and *D. tubericola* caused a stem-end rot of watermelons. It is possible that only one fungus is being dealt with here, as the organisms pass readily from one host to another. However, it has not been demonstrated that the Diplodia on corn and that on sweet potatoes have *P. rhodina* as their perfect stage. In view of this fact the wisest course at present seems to be to consider *D. frumenti* on corn as a species distinct from *D. tubericola* and *P. rhodina*.

Germination of the spores

Spores of *D. frumenti* were germinated in tap water in drop cultures exposed to the following temperatures Centigrade: 5°, 8°, 15°, 20°, 23°, 25°, 28°, 32°, 35°, and 39°. The daily fluctuations in these temperatures were not more than plus or minus one degree. The percentage germination at each temperature at the end of four hours is given in table 2. When the same cultures were held at the various temperatures for six days, the spores germinated at temperatures as low as 8° C.

TABLE 2.—*Percentage germination of spores of Diplodia frumenti exposed to different temperatures for four hours*

Temperatures in degrees Centigrade	5	8	15	20	23	25	28	32	35	39
Percentage germination	0	0	0	0	11	28	40	32	27	11

Growth on culture media

Diplodia frumenti was grown on sterilized apple, sweet-potato, and orange plugs and sterilized wheat and corn kernels. Pycnidia were formed by the organism on all these media except the orange plugs. When grown on corn meal, carrot, oatmeal, glycerine, lactose, potato-dextrose, and malt-extract agars at room temperature, the fungus formed dark brown stromatic masses of mycelium on the surface of these media and produced pycnidia on all except the lactose and malt-extract agars.

Relation of temperature to mycelial growth

A series of triplicate plates of potato-dextrose agar were inoculated in the center with uniform squares of a young agar culture of the organism and incubated for 48 hours at temperatures ranging from 5° to 39° C. Maximum growth, as indicated by the diameters of the colonies in table 3, occurred between 25° and 32° C. The fungus grew slightly at 39° C., and the lower limit at which growth occurred was 15° C.

TABLE 3.—*Relation of temperature to the mycelial growth of Diplodia frumenti on 2 per cent potato-dextrose agar*

Time of reading	Diameter of colonies in centimeters at temperatures expressed in degrees Centigrade									
	5	8	15	20	23	25	28	32	35	39
24 hours	0	0	0.5	1.7	2.5	3.7	4.2	5.0	1.1	trace
48 hours	0	0	1.8	4.3	6.5	8.3	8.7	9.0	1.7	0.3

Relation of hydrogen-ion concentration to growth

Diplodia frumenti was grown on a 2 per cent potato-dextrose agar adjusted to different hydrogen-ion concentrations. Fifteen cubic centimeters of the agar were placed in each of 120 test tubes, and, after sterilization, the adjustment of the pH concentration of each tube was made by adding the required number of drops of 2N, N/1, N/3, HCl, and N/1 NaOH. The amount of acid and alkali required to adjust the media to the different concentrations was determined with the Youden Hydrogen-Ion Concentration Apparatus. Petri plates were prepared in triplicate for each reaction, inoculated in the center with uniform bits of fungous mycelium, and exposed at a constant temperature of 30° C. for 48 hours. According to the diameters of the colonies given in table 4, the fungus made about the same rate of growth at pH concentrations ranging between 4.9 and 7.6. The organism grew well at pH 8.2, but growth was inhibited at pH 1.9, the lower limit.

TABLE 4.—*Relation of hydrogen-ion concentration of 2 per cent potato-dextrose agar to the mycelial growth of Diplodia frumenti at 30 C.*

pH	Average diameter of colonies in centimeters after	
	24 hours	48 hours
1.9	0	0
2.9	0	trace
3.5	1.8	4.5
4.6	2.3	5.1
4.9	2.8	5.6
5.2	3.0	5.6
6.0	2.8	5.7
7.1	3.2	5.8
7.6	3.2	5.8
8.2	1.5	4.5

Pathogenicity on corn

Ears of corn in the soft-dough stage were inoculated with *D. frumenti* by the following methods: (1) A kernel of corn on which a pure culture of the fungus was growing was inserted into a wound made in the husks and pressed down in contact with the kernels; (2) a water suspension of spores was sprayed on the exposed tips of ears; and (3) a water suspension of spores was hypodermically injected into the husks of ears near the butt end. As noted in table 5, ears were infected when inoculated by each method. The diseased ears had some or all of their kernels severely rotted and blackened by the organism which was recovered in pure culture when infected kernels were plated on agar. The data show that the fungus may gain entrance to the ears through exposed tips and wounds. Ears in the field often are diseased only at the butt ends. This indicates that the spores lodge in the cavities formed by the husks and ear sheath at the butt, where they germinate, and the fungus grows into the ear.

TABLE 5.—*Results obtained by inoculating ears of corn in the soft-dough stage with mycelial cultures and spore suspensions of Diplodia frumenti*

Method of inoculation	Number of ears inoculated	Number of ears infected	Number of ears on which pycnidia appeared
Mycelial cultures inserted into wounds in husks	66	42	7
Spore suspension sprayed on exposed tips	81	11	6
Spore suspension injected into husks near butt end	79	18	9

Healthy corn seedlings with roots from one to two inches long were set out in sterilized white lake sand and in sand sterilized and inoculated with a pure culture of *D. frumenti*. At the end of two weeks the roots of seedlings grown in sand inoculated with the organism were severely rotted and blackened and the tops of the plants were either wilted or dead. Seedlings in the noninoculated sand were healthy. In this experiment the amount of inoculum was greatly in excess of what normally occurs in the field. Consequently, the test indicates only the parasitic capabilities of the fungus and not its pathogenicity under normal field conditions.

Pathogenicity on other plants

The pathogenicity of *D. frumenti* was tested on oranges, grapefruits, sweet potatoes, cotton bolls, and watermelons. Inoculations were made

under sterile conditions by inserting mycelium from pure culture into wounds made in each fruit with a sterilized scalpel. As shown in table 6, all these fruits were rotted by the organism, thus demonstrating that *D. frumenti* is not confined to corn but can cause a typical Diplodia rot of oranges, grapefruits, sweet potatoes, cotton bolls, and watermelons.

TABLE 6.—*Results of inoculating oranges, grapefruits, sweet potatoes, cotton bolls, and watermelons with mycelium of Diplodia frumenti*

Fruits inoculated	Number inoculated	Number rotted	Number noninoculated (checks)	Number rotted (checks)
Oranges	8	8	12	0
Grapefruits	3	3	2	0
Sweet potatoes	14	10	15	0
Cotton bolls	22	22	24	3[a]
Watermelons	3	3	2	0

[a] The cotton was inoculated in the field and the 3 rotted bolls of the noninoculated checks were naturally infected.

In another test, mycelium from a pure culture of *D. frumenti* was inserted into wounds made in the bark of young orange twigs. The wounds were wrapped with absorbent cotton which was moistened each day. At the end of three weeks gummosis and necrosis of the twigs had occurred in the region of the wounds and at ten weeks pycnidia containing mature spores had been formed. The noninoculated wounds that served as checks healed and no symptoms of Diplodia infection were evident.

Overwintering

Diplodia frumenti survives the winter in Florida on old plant débris in the field, for ear infection has been obtained from spores collected from old stalks left in the field until June. It also lives over as dormant mycelium in the seed.

SUMMARY

A hitherto unknown Diplodia ear-rot of corn has been found in Florida. The stalks also are attacked. The symptoms of the disease and morphology of the fungus are described.

The causal organism agrees closely with *Diplodia frumenti* E. and E. and is referred to that species. Cross inoculations showed that *D. tubericola*, *D. natalensis*, and *D. gossypina* cause a rot of ears of corn similar to that caused by *D. frumenti*, and all forms cause a similar rot of oranges, grapefruit, sweet potatoes, cotton bolls, and watermelons. The four

organisms resemble each other in their imperfect stages. *D. natalensis* and *D. gossypina* are synonymous and have *Physalospora rhodina* as the perfect stage. *D. frumenti* and *D. tubericola* are tentatively considered two distinct species because their perfect stages are unknown.

Spores of *D. frumenti* germinate best at temperatures ranging between 25° and 32° C. The fungus grows well on standard culture media. The temperatures at which maximum growth occurs on potato-dextrose agar range between 25° and 32° C., and the pH reaction most favorable for growth lies between pH 4.9 and 8.2.

Diplodia frumenti enters the ears through the exposed tips and wounds and by growing into the ears at the butt ends. The organism hibernates in old cornstalks in the field and as dormant mycelium in the seed.

LITERATURE CITED

1. EDDINS, A. H. Dry rot of corn caused by *Diplodia macrospora* Earle. Phytopath. **20**: 439–448. 1930.
2. ELLIS, J. B., and B. M. EVERHART. New species of fungi from various localities. Jour. Mycol. **2**: 99–104. 1886.
3. FAWCETT, H. S., and O. F. BURGER. A gum-inducing Diplodia of peach and orange. Mycologia **3**: 151–153. 1911.
4. HARTER, L. L., J. L. WEIMER, and J. M. R. ADAMS. Sweet-potato storage-rots. Jour. Agr. Res. **15**: 337–368. 1918.
5. MEIER, F. C. Watermelon stem-end rot. Jour. Agr. Res. **6**: 149–152. 1916.
6. STEVENS, NEIL E. Two species of Physalospora on citrus and other hosts. Mycologia **18**: 206–217. 1926.
7. TAUBENHAUS, J. J. The probable non-validity of the genera Botryodiplodia, Diplodiella, Chaetodiplodia, and Lasiodiplodia. Amer. Jour. Bot. **2**: 324–331. 1915.

THE IDENTITY OF THE POTATO BLACKLEG PATHOGENE[1]

J. G. Leach

The question of the correct name to be applied to the blackleg pathogene has been the subject of much discussion and the cause of considerable confusion. Frank (3) in 1899 was perhaps the first to publish a recognizable description of the disease and to attribute it to a definite bacterial pathogene. He described briefly a Micrococcus, which he called *M. phytophthorus*. Since no later workers have found a Micrococcus to be pathogenic to potatoes and since abundant evidence has been presented to show that blackleg is due to a Bacillus, it is to be concluded that Frank's organism was not the true pathogene.

In 1902, van Hall (4) described a Bacillus as the pathogene and named it *Bacillus astrosepticus*. Shortly before this, Appel (1) reported the pathogene as *Bacillus phytophthorus* but did not publish a complete description until 1903 (2). Harrison (6), in 1907, published a detailed description of blackleg as it occurred in Canada. He described the pathogene as a Bacillus slightly different from those already reported and named it *B. solanisaprus*. In 1911, Pethybridge and Murphy (16) attributed the disease in Ireland to *B. melanogenes*, which they found to differ slightly from the previously described organisms. Morse (14), in 1917, published the results of comparative studies of *B. atrosepticus* van Hall, *B. solanisaprus* Harrison, *B. melanogenes* Pethybridge and Murphy, and three cultures isolated from diseased potatoes in Maine. Although some slight differences were found, such as "slight variations in size, shown more particularly by *B. solanisaprus* and IIIA, and the production of a slight viscidity on different kinds of media shown by *B. solanisaprus* and *B. melanogenes*," he concluded that they "should be classed as one species or at the most, strains of the same species." On the grounds of priority the name *B. astrosepticus* van Hall was held to be valid. Unfortunately, an authentic virulent culture of *B. phytophthorus* Appel was not included in the comparison.

Jennison (7), however, has repeated and extended this phase of Morse's work, comparing twelve strains of the pathogene, including a virulent culture of Appel's original strain of *B. phytophthorus*, as well as cultures of the other three so-called "species." Jennison agreed with Morse that the slight differences were not sufficient for species characterization. He also agreed with Morse that the pathogene should bear the name *B. astro-*

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septicus van Hall. Smith (19), however, preferred to use the name *B. phytophthorus* Appel.

Paine and Chaudhuri (15), in 1923, using a culture of *B. astrosepticus* isolated and identified by Paine in 1917 and one of *B. solanisaprus* obtained in the same year from the American Museum of Natural History, found the two to differ in several important respects, such as resistance to heat, type of decay on potato tubers and stems, and gelatine liquefaction.

St. John-Brooks, Nain, and Rhodes (17), in 1925, compared several strains of *B. carotovorus,* two strains of *B. phytophthorus,* and one of *B. solanisaprus.* All strains except one of *B. carotovorus* produced identical reactions in sugar media and behaved alike on other media but were found to differ serologically.

In the course of a study of the relation of the seed-corn maggot and potato blackleg, the writer had occasion to isolate a large number of cultures of bacteria from the different stages of the insect, from the soil, and from potatoes affected with blackleg. Many of the cultures proved pathogenic to potato tubers and stems. Since *B. carotovorus* Jones is known to be pathogenic to potatoes and since the seed-corn maggot is known to breed in plants which are recognized hosts for *B. carotovorus,* it was expected that some of the cultures would prove to be this organism. Therefore, an effort was made to distinguish *B. carotovorus* from the blackleg pathogene, but it was found impossible to do so. Although there were slight variations in minor cultural characteristics among the cultures isolated, they all had the same primary characteristics as indicated by the index number[2] 5010–32120–0111. For closer comparison, a culture of *B. carotovorus* Jones was obtained from Professor A. B. Massey. This was said to be a subculture of Jones's original type culture designated as 3a (12). This culture was found to be identical with an authentic culture of the blackleg pathogene (*B. phytophthorus* Appel I) obtained by the writer from E. F. Smith, in so far as primary characteristics were concerned, although there were a few differences in minor cultural characters. The most pronounced difference was the production of less black pigment by *B. carotovorus* Jones 3a on potato tubers and stems. When this character was used in an attempt to identify the unknown cultures so many intermediate forms were found that it was impossible to do so. Massey (12) was able to distinguish *B. phytophthorus* and *B. carotovorus* on the basis of the production of acid by the latter on 5 per cent alcohol agar. This test was used by the writer, also, but numerous other pathogenic cultures isolated from rotting carrots, cabbage, celery, onions, and iris, otherwise answering the description of *B. carotovorus,* did not show this property. Since there appeared

[2] The descriptive chart of the Society of American Bacteriologists.

to be strains of *B. carotovorus* that did not possess this property it was felt that the test could not be relied on for identifying the species.

Kotila and Coons (10) showed that *B. astrosepticus* produced a toxic substance that would diffuse through a colloidal membrane and cause potato leaves to wilt. They also showed that 5 cc. of a broth culture of *B. astrosepticus* added to a nutrient solution containing the uninjured roots of a potato plant would not infect the plant but would produce a substance that was toxic to it. They conclude that, "*B. astrosepticus* seems to possess the power of producing a toxic substance which affects potato cells so as to allow blackening, probably oxidation, to ensue, while *B. carotovorus* which softens potato tissues under some conditions without blackening, does not possess this power."

These authors, however, did not report any experiments to show whether *B. carotovorus* would likewise produce a toxic substance. The writer, therefore, made experiments both with *B. carotovorus* and *B. astrosepticus*. It was found that both cultures produced a toxic substance that wilted cut leaves and caused the yellowing and ultimate death of plants grown in water cultures to which 5 cc. of broth cultures had been added. This property therefore could not be used in identifying the cultures.

The similarity between the blackleg pathogene and *B. carotovorus* Jones, the cause of a soft rot of many succulent plants has been recognized by many workers. After comparing several cultures of bacteria causing soft rots, Smith (19), stated that *B. phytophthorus* was "not sufficiently distinguished from *Bacillus carotovorus* Jones, which name is earlier."

Harding and Morse (5), working in collaboration with Jones, made a detailed physiological study of forty-three strains of bacteria causing soft rot of plants. They found that the strains could be separated into six groups on the basis of their action on dextrose, lactose, and saccharose. They did not consider these differences sufficient to justify the recognition of six different species but regarded the cultures as somewhat variant strains of a single species. Jones (9) also made a study of the enzyme activity of the same strains studied by Harding and Morse. He found considerable variation in this respect and presented the results as "contributing further evidence to the general likeness of these strains and partly as emphasizing the minor variations which we believe must always be expected to occur with different bacterial strains, even of the same so-called species." These differences, however, are equally great, as the differences on which the various "species" of the blackleg pathogene have been distinguished from each other and from *B. carotovorus*. To facilitate comparison the index numbers of the principal soft-rotting bacteria as determined by various workers have been assembled in table 1.

From this summary it will be noted that the chief point of difference between *B. carotovorus* 3a and the various "species" of the blackleg pathogene is in their diastatic properties. Jones (9), Harding and Morse (5), Smith (19), and Mehta (13) report *B. carotovorus* Jones as possessing diastatic properties usually designated as weak. Harrison (6), Morse (14), Mehta (13), and Pethybridge and Murphy (16) attribute diastatic action to the blackleg pathogene also, while Jennison (7), Shapovalov and Edson (18), and van Hall (4) found no action on starch by the latter organism. The present writer when comparing the two organisms side by side was unable to obtain a positive diastatic reaction with either. A study of the literature shows that these differences can, in all probability, be attributed to differences in method. Those workers reporting positive diastatic action have generally used the old method in which the organism is cultured on potato plugs and later treated with an alcoholic solution of iodine, decolorization of the iodine solution being considered evidence of diastatic action. Later workers generally have used the starch-agar method recommended by the Society of American Bacteriologists. By this method both cultures react negatively.

A careful comparison of the data published by various workers dealing with the various soft-rotting bacteria reveals the following facts:

1. The differences between the various "blackleg pathogenes" as described by different workers are as great as or greater than those which are supposed to distinguish them from *B. carotovorus*.
2. The differences found by various workers using, presumably, the same organisms are as great as or greater than those used for distinguishing the blackleg pathogenes from *B. carotovorus*.
3. The differences between the six groups of soft-rotting bacteria described by Harding and Morse, of which *B. carotovorus* is the type, are greater than those which are used for distinguishing the blackleg pathogenes from them.
4. The revised description of the blackleg pathogene as given by Jennison (7) agrees in all essential details with the recognized characteristics of *B. carotovorus*.

Many workers have observed the morphologic and physiologic similarity of these organisms, but they usually have considered slight physiologic differences sufficient for differentiating two separate species. The recognition of these slight physiologic differences by different specific names has apparently carried with it an implication of parasitic differences. Jones (8), in his first paper on *B. carotovorus*, reported unsuccessful attempts to infect potato tubers but, in a footnote, states that later inoculations were successful. Smith (19) later showed that this organism was readily pathogenic to potato tubers and stems. However, it was not until 1926

TABLE 1.—*Summary of the primary characteristics (represented by index numbers) of various soft-rotting bacteria, as described by various workers*

Organism	Index number	Authority	Organism	Index number	Authority
B. carotovorus, Jones 3a	5011[a]–32120–1111	Jones	*B. phytophthorus* Appel	5U1U–32120–UUU1	Appel
do	5010–32120–1111	Harding and Morse	do	5010–32120–U111	Smith
do	5010–32120–1111	Smith	do	501U–3U120–U111	Stapp
			do	5010–32120–1111	Mehta
do	5010–32120–1111	Mehta	do	5010–32120–0111	Shapovalov and Edson
do	5010–32120–0111	Leach	do	5010–32120–0111	Jennison
do, Vermont XLVIII	5010–32120–1112	Harding and Morse	do	5010–32120–0111	Leach
do, Vermont C	5010–32120–1121	do	*B. atrosepticus* v. Hall	5010–32120–0121	van Hall
do, Vermont LIV	5010–32120–1211	do	do	5010–32125–1111	Morse
do, Potter's Bacillus	5010–32120–1212	do	do	5010–32120–1121	Harrison
B. solanisaprus, Harrison	5010–32120–1212	Harrison	do	5010–32120–0111	Jennison
do	5010–32125–1111	Morse	*B. melanogenes* P and M	5010–32110–1111	Pethybridge and Murphy
do	5010–32120–0111	Jennison	do	5010–32125–1111	Morse
do	5010–32120–0111	Leach	do	5010–32120–0111	Jennison

[a] Figures in bold face type indicate points of difference when compared with the findings of the writer and the revised description of the blackleg pathogene by Jennison.

(11) that a natural infection of potato tubers due to *B. carotovorus* was reported. In so far as the present writer has been able to learn, no one has yet recorded in literature a natural primary infection of potato stems due to *B. carotovorus*. Is this due to the inability of *B. carotovorus* to infect the potato plant under natural conditions, or have we been deceived by the names applied to these organisms and assumed that all soft rot of carrots is due to *B. carotovorus* and that all soft rot of potato stems is due to *B. phytophthorus?*

If it is true that *B. carotovorus* is pathogenic to potato tubers and stems and that the blackleg pathogene is pathogenic to carrots and other vegetables, it does not seem reasonable that they should be so closely specialized in their attack on these plants in nature. In studying this question several strains of virulent soft-rotting bacteria were isolated from carrots, onions, cabbage, celery, iris, and potato tubers and stems. Cross inoculations were made and all of the cultures, without exception, were found strongly parasitic on all of the hosts. Some slight differences were observed in minor cultural characters, but all the cultures were identical in so far as the major physiological tests were concerned. They all had the index number 5010–32120–0111. Some slight differences in color of the decayed tissues were observed, but this character was very variable, being influenced by the variety of host plant used, the age of tissue, the humidity of the incubation chamber, etc. It was quite evident, however, that certain of the cultures differed in amount of pigment produced under the same conditions.

One of the most consistent differences between *B. carotovorus* and the blackleg pathogene as reported by various writers is the so-called "inky black" discoloration of tissues affected with blackleg, which is absent in tissues affected with *B. carotovorus*. The culture used by the writer, designated as *B. carotovorus* Jones 3a, was definitely characterized by the production of less pigment than most of the cultures studied. On the other hand, several cultures obtained from carrots, onions, and cabbage produced more pigment than certain cultures isolated from typical blackleg plants. It should be stated here also that the inky black discoloration so frequently mentioned in descriptions of blackleg is not always characteristic of the disease as it occurs in Minnesota. Potato tissue killed by almost any organism will turn black when thoroughly dried and oxidized, but tissues while in the process of decay by the blackleg pathogene are more frequently dull brown than inky black. The black color usually appears after the tissues have become relatively dry.

Lacey (11) compared *B. carotovorus* with several other soft-rotting organisms and concluded that "cultural, pathological, and serological tests all show that a close relationship exists between the three species, *B.*

carotovorus, *B. solanisaprus*, and *B. phytophthorus*, but there are nevertheless sufficiently marked and constant differences to warrant their continued separation into different species." Stapp (20), on the other hand, has compared many strains of the blackleg pathogene with *B. carotovorus* and other similar organisms. He classified them into five groups on the basis of serological and physiological differences but proposed to include all five groups in one common and uniform "*Bacillus phytophthorus*-group." Inasmuch as our concept of what constitutes a species among the bacteria is extremely vague, it appears to be only a matter of opinion as to whether a distinct specific name should be given to each of these soft-rotting organisms. The writer is inclined to favor the idea proposed by Stapp of including all in one species, but on the basis of priority it appears that *B. carotovorus* would be the correct name because *B. carotovorus* was described two years before *B. phytophthorus*.

Stapp (20) recognizes that the name *B. carotovorus* is earlier but feels that it should not be used because:

". . . 1. Jones seinem Bakterium einen von der Kulturpflanze (der Karotte), von der er den Erreger isoliert hatte, abgeleiteten Namen gegeben hat und garnicht wusste, dass dieser Mikroorganismus auch eine wirtschaftlich bedeutend wichtigere Krankheit hervorzurufen imstande ist und (dass) 2. der allergrösste Prozentsatz der zahlreichen von mir vergleichend geprüften Bakterienstämme serologisch völlig mit dem Originalstamm von *Bac. phytophthorus* Appel übereinstimmt. . . ."

The writer has no desire to quibble nor to add to the great amount of confusion already existing in regard to the name of the blackleg pathogene, but it does not appear to him that the above reasons are sufficient for disregarding the priority of the name *B. carotovorus* Jones.

At any rate, it must be recognized that potato blackleg is a bacterial soft rot and may be caused by several closely related strains of bacteria and that these same bacteria may cause a soft rot of many other plants. If we separate the "soft rot" group of bacteria into seperate species on the basis of minor physiological differences we must recognize that potato blackleg can be caused by *B. carotovorus* as well as by *B. phytophthorus*.[3] In other words, potato blackleg is considered to be nothing more than "soft rot" of the potato. The distinctive characters of the disease are due primarily to the nature of the host plant rather than to the causative agent. The potato being propagated by tubers is more subject to infection than

[3] *Bacillus aroideae* Townsend has not been considered in this discussion. It has been isolated only a few times in the course of this work. There appears to be some justification in considering this organism as a distinct species on the basis of its reaction on sugar media and of other cultural characteristics. It is, however, pathogenic to potatoes.

most plants propagated by seed. The transmission of the organism through infected seed pieces and the tendency of decayed potato tissue to form a black pigment upon oxidation have been factors contributing to the distinctive character of the disease when affecting the potato.

SUMMARY

The similarity of the bacteria causing potato blackleg to *B. carotovorus* causing soft rot of carrots and other vegetables led to a comparative study of numerous strains of pathogenic bacteria isolated from various host plants. A comparison of the morphologic, physiologic, and parasitic characteristics of these cultures showed that there is little justification for recognizing different species within the group. All of the bacteria studied were equally pathogenic to potatoes and a large group of succulent vegetables and were identical in so far as primary characters are concerned. There appears, however, to be a fairly large number of strains varying in minor characteristics, but there is no evidence of parasitic differences between those isolated from potatoes and those isolated from other vegetables. The conclusion is reached that blackleg is nothing more than soft rot of potato and that the bacteria previously designated as *B. phytophthorus* Appel, *B. astrosepticus* van Hall, *B. solanisaprus* Harrison, and *B. melanogenes* Peth. and Murph. are merely strains of the earlier-described species, *B. carotovorus* Jones.

UNIVERSITY FARM,
SAINT PAUL, MINN.

LITERATURE CITED

1. APPEL, O. Der Erreger der "Schwarzbeinigkeit" bei den Kartoffeln. Ber. Deut. Bot. Gesell. 20: 128–129. 1902.
2. ————. Untersuchungen über die Schwarzbeingkeit und die durch Bakterien hervorgerufene Knollenfäule der Kartoffel. Arb. biol. Abt. Land.-u. Forstwirt., K. Gsndhtsamt. 3: 364–432. 1903.
3. FRANK, A. B. Die Bakterienkrankheiten der Kartoffeln. Centralbl. f. Bakt. Abt. II. 5: 98–102, 134–139. 1899.
4. VAN HALL, C. J. J. Bijdragen tot de kennis der Bakterieele Plantenziekten. Inaug. Diss. Univ. Amsterdam. Cooperative Druckerij-Vereeniging "Plantyn." Amsterdam. 198 pp. 1902.
5. HARDING, H. A., and W. J. MORSE. The bacterial soft-rots of certain vegetables. Vt. Agr. Exp. Sta. Bul. 147. 1909.
6. HARRISON, F. C. A bacterial rot of the potato, caused by *Bacillus solanisaprus*. Centralbl. f. Bakt. II. 17: 34–39, 120–128, 166–174, 384–395. 1907.
7. JENNISON, H. M. Potato blackleg with special reference to the etiological agent. Ann. Mo. Bot. Gard. 10: 1–72. 1923.
8. JONES, L. R. A soft rot of carrot and other vegetables caused by *Bacillus carotovorus* Jones. Vt. Agr. Exp. Sta. Ann. Report 13: 299–332 (1899–1900). 1901.

9. ————. The bacterial soft rots of certain vegetables. II Pectinase, the cytolytic enzyme produced by *Bacillus carotovorus* and certain other soft-rot organisms. New York (Geneva) Agr. Exp. Sta. Tech. Bul. 11: 291–368. 1909.

10. KOTILA, J. E., and G. H. COONS. Investigations on the blackleg disease of potato. Mich. Agr. Exp. Sta. Tech. Bul. 67: 1925.

11. LACEY, MARGARET S. Studies in Bacteriosis, XIII. A soft rot of potato tubers due to *Bacillus carotovorus* and a comparison of the cultural, pathological and serological behavior of various organisms causing soft rots. Ann. Appl. Biol. 13: 1–11. 1926.

12. MASSEY, A. B. A study of *Bacillus aroideae* Townsend, the cause of a soft rot of tomato, and *B. carotovorus* Jones. Phytopath. 14: 460–477. 1924.

13. MEHTA, MANECK M. A physiological study of the common bacteria of air, soil and water with a view to their exact diagnosis. Ann. Appl. Biol. 12: 330–358. 1925.

14. MORSE, W. J. Studies on the blackleg disease of the potato with special reference to the relationship of the causal organisms. Jour. Agr. Res. 8: 79–126. 1917.

15. PAINE, SYDNEY G., and H. CHAUDHURI. The blackleg disease of the potato. On the relationship of *Bacillus atrosepticus* and *Bacillus solanisaprus*. Phytopath. 13: 359–361. 1923.

16. PETHYBRIDGE, G. H., and P. A. MURPHY. A bacterial disease of the potato plant in Ireland. Proc. Roy. Irish Acad. Sect. 29 B: 1–37. 1911.

17. ST. JOHN-BROOKS, R., K. NAIN, and MABEL RHODES. The investigation of Phytopathogenic bacteria by serological and biochemical methods. Jour. Path. and Bact. 28: 203–209. 1925.

18. SHAPOVALOV, M., and H. A. EDSON. Blackleg potato tuber-rot under irrigation. Jour. Agr. Res. 22: 81–92. 1921.

19. SMITH, E. F. An introduction to bacterial diseases of plants. 688 pp. W. B. Saunders Co., Philadelphia and London. 1920.

20. STAPP, C. Die Schwarzbeinigkeit und Knollennassfaüle der Kartoffel. Arb. Biol. Reichsanst. Land-u.-Forstw. 16: 643–703. 1929.

PHYTOPATHOLOGICAL NOTES

Ineffective nature of iodine dust as a fungicide against Tilletia caries.—In 1928 it was suggested to the writer that iodine dust, mixed with an infusorial earth, might be useful as a fungicide against bunt of wheat. In order to test this chemical the following plots were laid down: Little Joss wheat was contaminated with bunt spores from the variety Victor at a rate, approximately, of one part of bunt balls to twenty-five parts of wheat. This sample of wheat was divided into ten portions. Of these two were treated with copper carbonate dust, two with 1½ per cent of iodine dust, two with 3 per cent of iodine dust, and two with 5 per cent iodine dust, and the remaining two were not treated. The treated samples were dusted in each case at the rate of 2 ozs. of dust to the bushel of wheat. Five plots were sown in the winter, 28th of November, 1928, and five in the spring, 15th of March, 1929. Each of the winter-sown plots consisted of twelve rows, a row being ten feet; but each spring-sown plot consisted of three ten-foot rows. At harvest a total head count was made and the percentage of bunted heads determined. The results of these experiments are given in table 1. From these results it appears that iodine dust is of no use as a fungicide for *Tilletia caries*. It is stated, however, by Sayre and Thomas[1, 2] that excellent results were obtained against oat smuts by the use of iodine vapor dust, made by mixing finely ground solid iodine with infusorial earth, the dust containing 5 per cent by weight of iodine. On the other hand, Horsfall[3] states that under the conditions of his Ithaca experiments, iodine dust was ineffective. Since these American results are antagonistic, this writer thought it would be of interest to communicate the results of his experiments on the use of iodine dust against bunt in wheat.—W. A. R. Dillon Weston, M.A., School of Agriculture, Cambridge, England.

[1] Sayre, J. D., and R. C. Thomas. New dust treatment for oats smuts. Science n. s. **66**: 398. 1927.

[2] ————. New dust treatments for oat smuts. Phytopathology **18**: 189. 1928.

[3] Horsfall, J. G. Dusting seed for oat smuts. Phytopathology **19**: 173–175. 1929.

TABLE 1

Winter-sown Plot No.	and variety	Treatment	Percentage of bunted heads
1586	Little Joss contaminated	2 ozs. of	2
1587	with Victor bunt	copper carbonate	0
1588		to the bushel of	4
1589		wheat	Less than 1
1590			1
1591			0
1592			0
1593			0
1594			0
1595			0
1596			0
1597			3
1598	Little Joss contaminated	2 ozs. of iodine dust,	9
1599	with Victor bunt	3 per cent, to the	11
1600		bushel of wheat	12
1601			13
1602			4
1603			13
1604			9
1605			4
1606			3
1607			12
1608			12
1609			17
1610	Little Joss contaminated	2 ozs. of iodine dust,	15
1611	with Victor bunt	1½ per cent, to the	11
1612		bushel of wheat	8
1613			16
1614			14
1615			7
1616			19
1617			5
1618			14
1619			7
1620			9
1621			15
1622	Little Joss contaminated	2 ozs. of iodine dust,	17
1623	with Victor bunt	5 per cent, to the	6
1624		bushel of wheat	3
1625			8
1626			15
1627			17

TABLE 1—(*Continued*)

Plot No.	Variety	Treatment	Percentage of bunted heads
Winter-sown			
1628			8
1629			17
1630			5
1631			14
1632			4
1633			15
1634	Little Joss contaminated	Nontreated	2
1635	with Victor bunt		15
1636			8
1637			8
1638			11
1639			13
1640			12
1641			14
1642			16
1643			20
1644			8
1645			16
Spring-sown			
2175	Little Joss contaminated	2 ozs. iodine dust,	0
2176	with Victor bunt	1½ per cent, to the	0
2177		bushel of wheat	2
2178	Little Joss contaminated	2 ozs. iodine dust,	0
2179	with Victor bunt	3 per cent, to the	0
2180		bushel of wheat	0
2181	Little Joss contaminated	2 ozs. copper carbonate	0
2182	with Victor bunt	dust to the bushel	Less than 1
2183		of wheat	0
2184	Little Joss contaminated	Nontreated	0
2185	with Victor bunt		Less than 1
2186			2
2187	Little Joss contaminated	2 ozs. iodine dust,	11
2188	with Victor bunt	5 per cent, to the	6
2189		bushel of wheat	3

A Bacterial Disease of Tung-Oil Tree.—In October, 1929, the writer observed for the first time a prominent spotting of the leaves of the tung-oil, or wood-oil, tree (*Aleurites fordi* Hemsl.)in southern Georgia. The spot was readily recognized as bacterial in character because of the immense number of bacteria in young and moderately old lesions and of the almost total absence of other organisms. This determination was confirmed by Lucia McCulloch, who examined material submitted to the Office of Mycology and Disease Survey, United States Department Agriculture. Necrotic lesions were present, also, on the trunk and petioles of young trees heavily affected with the leaf spot and are suspected to have the same origin.

The occurrence of this disease seems to be of considerable interest, inasmuch as there appear to be no bacterial diseases described for any of the five species listed for Aleurites (*A. fordi* Hemsl., *A. montana* (Lour) Wils., *A. cordata* R. Br., *A. moluccana* Willd. [syn., *A. triloba*], and *A. trisperma* Blanco [syn., *A. saponaria*]) in their native habitats or elsewhere; and no bacterial leaf spots for any of the related plants, *i.e.*, Euphorbiaceae, in this country. For conditions in Florida,[1] the belief has been expressed that *A. fordi*, on being introduced into the United States, brought none of its native insect pests or diseases with it, and, at that time, the plant in Florida showed no disease except the common root-knot disease (*Caconema radicicola*). A survey of tung-oil plantings in southwestern Georgia revealed the presence of the leaf spot, together with bark and petiole lesions, in each of the three nurseries inspected, and the leaf spot in all five orchard plantings visited, ranging in age from two to eight years, as well as in most of the scattered ornamental trees of various ages and locations. The oldest trees on which leaf spot was observed were isolated plants reported to be about thirty years old. The disease was prominent, however, and notably injurious in only one planting, *i.e.*, a thickly planted nursery of one season's growth containing comparatively rapidly growing, succulent trees. It seems, therefore, that the disease may not be necessarily very recent in its occurrence in the State or limited in its distribution.

In regard to the economic importance of the disease, it might be stated that in case the tung-oil industry in the Gulf States continues to increase in extent as it has during the past few years, it may prove of some importance.

The manner in which the disease is spread is not known. The fact, however, that it occurs in Georgia in nearly all plantings examined, suggests its probable occurrence on other common host plants or that the causal organism may be readily seed-disseminated.

The leaf spots are characteristic of the angular type of bacterial lesions on such organs, are decidedly delimited by veins (Fig. 1, A), brown to black on the upper side and yellowish brown on the lower surface, and are sur-

[1] Newell, W. The tung-oil tree in Florida. Fla. Agr. Exp. Sta. Bul. 171. 1924.

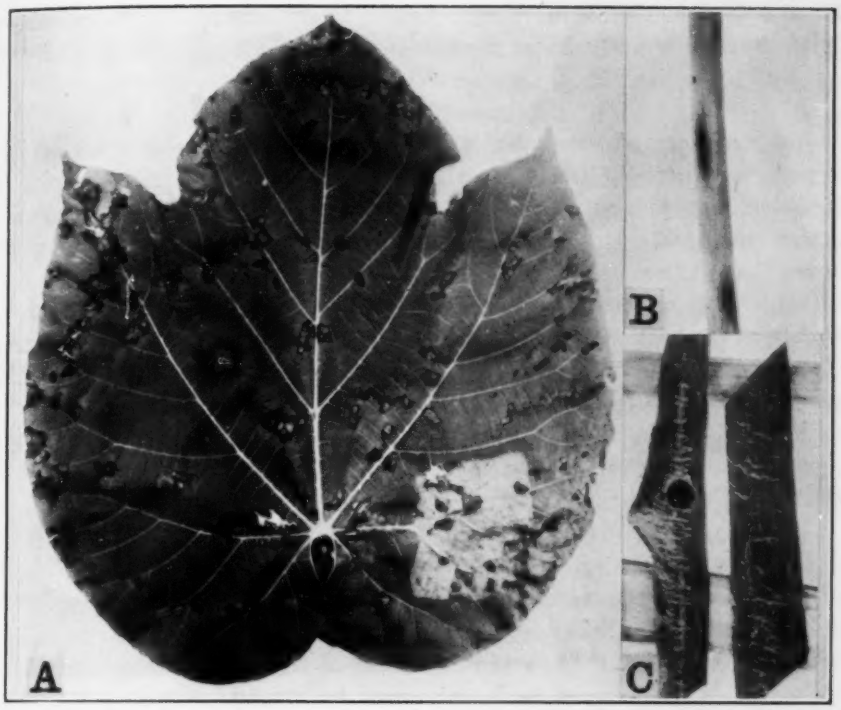

FIG. 1. A. Upper surface of tung-oil leaf showing characteristic spotting by the bacterial disease. Center of older lesions, reddish brown to yellowish brown in color. B. Lesions on a leaf petiole. C. Trunk bark of trees affected with the bacterial leaf spot. A, about ⅓ natural size. B and C, about natural size.

rounded by a slightly water-soaked, faintly yellowish halo of indefinite width. The lesions may coalesce to form large areas of dead, easily broken tissue, thus imparting a torn and ragged appearance to the leaf. The accompanying lesions on the petioles (Fig. 1, B) are long, and brown and extend into the cortex; those on the bark (Fig. 1, C) round to oblong, brown to black in color, extending into the wood.

In the heavily infected nursery planting referred to above, practically every tree in the three to four acre plot showed leaf infection, and many plants, notably those in certain scattered spots that appeared to represent points of primary infection, showed all of their leaves severely spotted and many badly torn. There was indication that secondary spread had been brought about by wind-blown rain and by workmen and teams during cultivation. Observations in general suggested that only relatively young tissues of well-nourished, rapidly growing young trees are likely to undergo severe injury during such a season. In no instance did trees four years old

or older show marked injury, regardless of health or rapidity of growth.—O. C. BOYD, *Formerly Plant Pathologist, Georgia State Board of Entomology.*

Some microscopic characters of the rot caused by Ganoderma Curtisii. *Ganoderma Curtisii* (Berk.) Murrill is very common on various species of hardwood trees along the southern Atlantic coast and extends inland as far as western Texas. It is easily identified by certain well-marked gross characters that differentiate it from all other species of Ganoderma. It frequently occurs on various species of oak, usually on the stumps and on old wounds at the base of living trees. It is one of the principal fungi that rot the roots of dead oak trees, thereby loosening them so that the stumps can be easily pulled from the soil.

Macroscopic characters of the rot. This fungus usually produces a soft indeterminate white rot with gross characters but little different from other similar rots of dead timber.

Microscopic characters. The characters herein given were taken from an early stage of the rot found in live oak, *Quercus virginiana* Mill.

When the vessels are treated with phloroglucin they stain a deep pink and show no indication of any corrosion of their bordered pits; no cellulose reaction obtains. The middle lamellae of the vessels are destroyed by the action of the fungus, as is evidenced by the isolation of the vessels when seen in tangential view. The middle lamellae in the large medullary rays are often dissolved, and the walls of the ray cells show yellowish to blue when treated with chloriodide of zinc. Many of the ray cells and tracheids stain purple, showing delignification.

The bordered pits of the tracheids are uniformly corroded into large round holes which often coalesce, causing the cell to rupture under stress along this line of weakness. The tangential walls of both large and small medullary rays are much perforated, with the holes often uniting. The cells in some of the large rays are entirely destroyed.

Hyphae are found in the cavities of the vessels, and yet their walls and pits are apparently not attacked. Many of the vessels are filled with hyaline hyphae two microns or less in size. Hyphae can also be seen permeating the tracheids, medullary rays, and other elements, running both longitudinally and transversely through them.—W. H. LONG, Forest Pathology, Bureau of Plant Industry, Albuquerque, N. Mex.

Polyporus dryadeus, a root parasite on white fir.—While on field work in what is known as Tejano Canyon in the Sandia Mountains near Albuquerque, New Mexico, the writer found many dead and dying trees of

white fir, *Abies concolor* Lindl. & Gord. Some of the trees were more than two feet in diameter, while others were small saplings. An examination of the diseased trees showed them attacked by some root parasite. Trees were found in all stages of the disease. Many were still alive but in a dying condition. In such cases all the larger roots were dead and in a rotten condition, while a few of the smaller surface roots were still alive but with their tips dead, and the disease was progressing along the roots toward the base of the tree. On some of the larger trees, sporophores were found attached to the collar of the tree. Old as well as fresh ones were found, some even in the sporulating stage. Specimens of various stages of the rot and of the sporophores were collected for study. These specimens agreed with *Polyporus dryadeus* in all essential characters, both gross and microscopic.

In a previous paper[1] the writer described this fungus as it occurs on oak and also called attention to a collection from the State of Washington on *Tsuga heterophylla* (Raf.) Sarg. It is now reported from another conifer, white fir. A collection has also been seen from California on this last host.

The old sporophores on white fir have the hymenium with tubes splitting into irregular areas. The surface of the pileus, in weathering, splits into irregular polygonal scale-like areas that separate slightly from each other. The fission is more or less parallel to the surface of the pileus. The weathered pileus is dark brown to almost black. The pileus of the fresh sporophores is typical of the eastern specimens of this fungus as it occurs on oak.

The setae were cat-claw shape, dark chestnut brown, thick-wall, 10 to 12 microns thick at the base by 25 to 30 microns long, averaging 11.5 by 26 microns. The spores were hyaline, obovate to globose, ranging from 7–8.5 by 7.5 to 10 microns, average 7.25 by 8.25 microns, usual size 7 by 8.5 microns. These characters of setae and spores agree with those found on oak.—W. H. LONG, Forest Pathology, Bureau of Plant Industry, Albuquerque, N. Mex.

[1] Long, W. H. *Polyporus dryadeus*, a root parasite on the oak. Jour. Agr. Res. 1: 239–250. 1913.